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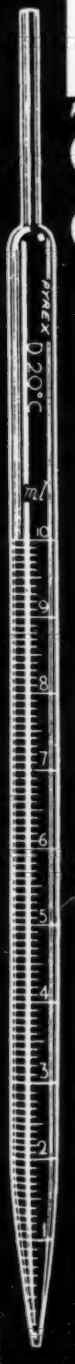


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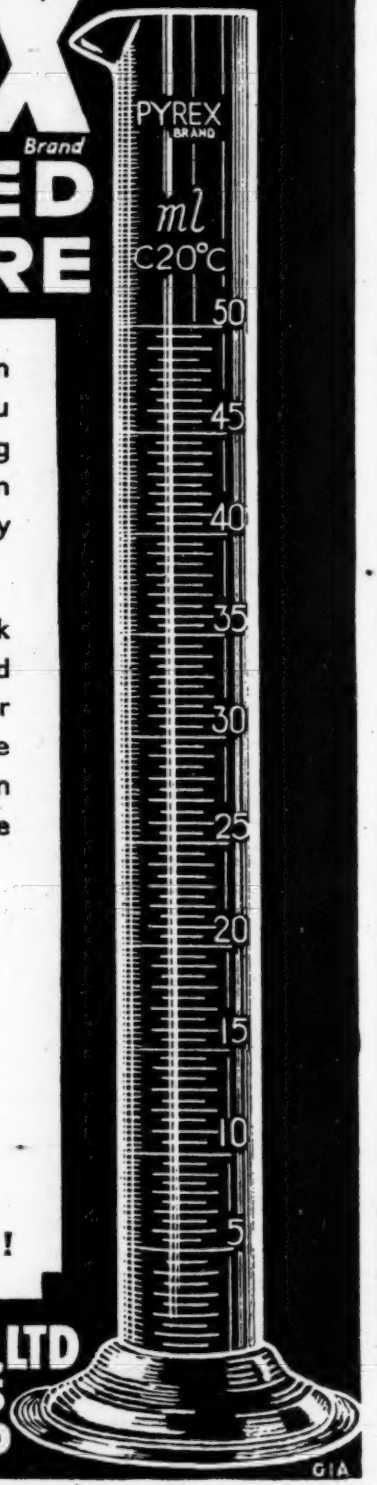


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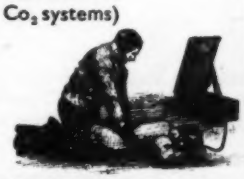

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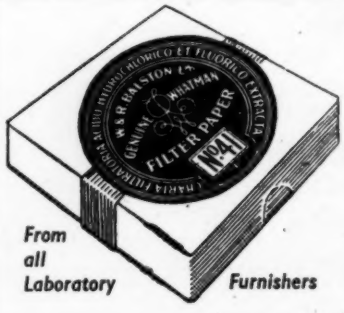


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By HERBERT E. WILLIAMS . . . . . 223

55. The Stability of Racemates. Mandelic Acid and Some of its Derivatives.
By W. ROGIE ANGUS and R. P. OWEN . . . . . 227

56. Aqueous Solubilities of *r*- and *l*-Mandelic Acids and Three *O*-Acyl-*r*-mandelic Acids.
By W. ROGIE Angus and R. P. OWEN . . . . . 231


### Organic.


49. The Stereochemical Relationships of the 9 : 10-Oxidostearic Acids and the 9 : 10-Dihydroxystearic Acids.
By D. ATHERTON and T. P. HILDITCH . . . . . 204

50. Physicochemical Aspects of Bacterial Growth. Part VIII. Growth of *Bact. Lactis Aerogenes* in Media containing Ammonium Sulphate or Various Amino-acids.
By R. M. LODGE and C. N. HINSHELWOOD . . . . . 208

51. Physicochemical Aspects of Bacterial Growth. Part IX. The Lag Phase of *Bact. Lactis Aerogenes*.
By R. M. LODGE and C. N. HINSHELWOOD . . . . . 213

52. Physicochemical Aspects of Bacterial Growth. Part X. Observations on the Variability of Growth Rate of *Bact. Lactis Aerogenes*.
By R. M. LODGE and C. N. HINSHELWOOD . . . . . 219

53. The Nitrosation of Phenols. Part XIX. The Three Cresols and their Methyl Ethers. Some Semicarbazide Reactions.
By HERBERT H. HODGSON and ERIC A. C. CROUCH . . . . . 221

57. The Interconversion of Hexœstrol and *iso*Hexœstrol.
By D. A. PEAK and W. F. SHORT . . . . . 232

58. Derivatives of 1 : 2 : 4 : 5-Tetrachlorobenzene. Part I. Nitro- and Amino-compounds.
By A. T. PETERS, F. M. ROWE, and D. M. STEAD . . . . . 233

59. Polyhalogeno-*o*-anisidines and their Derivatives.
By W. S. W. HARRISON, A. T. PETERS, and F. M. ROWE . . . . . 235

60. A Synthesis of Methacrylic Acid.
By THEODORE WHITE . . . . . 238

61. Polymethylbenzoylnaphthoic Acids.
By RICHARD H. MARTIN . . . . . 239

62. Synthesis of Some α-Acyltetronic Acids.
By WILSON BAKER, K. D. GRICE, and A. B. A. JANSEN . . . . . 241

63. Thiazinocyanines. Part II. Cyanines containing the Dihydro-1 : 3-thiazine Nucleus.
By FRANCES M. HAMER and RUSSELL J. RATHBONE . . . . . 243

NOTE.—The Effect of Heat on Mandelic Acid.
By W. ROGIE ANGUS and R. P. OWEN . . . . . 249


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# INDEX OF AUTHORS' NAMES.

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ANGUS, W. R., and Owen, R. P., 227, 231, 249.
Atherton, D., and Hilditch, T. P., 204.
Baker, W., Grice, K. D., and Jansen, A. B. A., 241.
Crouch, E. A. C. See Hodgson, H. H.
Grice, K. D. See Baker, W.
Hamer, F. M., and Rathbone, R. J., 243.
Harrison, W. S. W., Peters, A. T., and Rowe, F. M., 235.
Hilditch, T. P. See Atherton, D.
Hinshelwood, C. N. See Lodge, R. M.
Hodgson, H. H., and Crouch, E. A. C., 221.
Jansen, A. B. A. See Baker, W.
Lodge, R. M., and Hinshelwood, C. N., 208, 213, 219.
Martin, R. H., 239.
Owen, R. P. See Angus, W. R.
Peak, D. A., and Short, W. F., 232.
Peters, A. T., Rowe, F. M., and Stead, D. M., 233.
Peters, A. T. See also Harrison, W. S. W.
Rathbone, R. J. See Hamer, F. M.
Rowe, F. M. See Harrison, W. S. W., and Peters, A. T.
Short, W. F. See Peak, D. A.
Stead, D. M. See Peters, A. T.
White, T., 238.
Williams, H. E., 223.

In the words of highly placed colleagues ". . . the State has lost a very able and faithful servant," and ". . . he is as much a victim of the war as if he had fallen in the front line."

During his career, Burt received the following honours: Kaisar-i-Hind (India), 1912; M.B.E., 1919; C.I.E., 1930; Knight Bachelor, 1936. In 1935 he was awarded the Silver Medal of the Royal Society of Arts for his paper on "The Indian Sugar Industry." He became a Fellow of the Chemical Society in 1905.

In 1906, he married Beatrice Maud, daughter of Alfred Geary, of Bristol, who was a constant, devoted support and inspiration to him; and who, with a married daughter and two grandchildren, survives him.

R. S. FINLOW.

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## FRANCIS WILLIAM CLIFFORD.

**1880—1942.**

F. W. CLIFFORD was born in Bayswater on March 16th, 1880, the eldest son of Thomas Andrews Clifford, a pharmacist in business on his own account. In his early schooldays Clifford was the victim of a serious accident, of which the immediate results necessitated his spending a considerable part of his boyhood in a spinal carriage and the after effects restricted to some extent his activities for the rest of his life. His education could not be continued in the normal fashion and his future must have been a source of anxiety to his parents, but he seems to have possessed even in youth the cheerful courage that distinguished him in later life and he settled his own future by insisting, as soon as his age and physical condition permitted him to start work, in applying in 1894 for a post as a junior assistant in the Willesden Green Free Library. Three years later he moved to the Hampstead Central Library, where he remained until 1898, when he secured his first appointment to a special library, that of University College. He had by this time largely recovered from the worst effects of his accident, had educated himself by assiduous private study and had become familiar with all the routine work of running a library. He seems to have thoroughly enjoyed his job at University College. He was given opportunities to attend lectures and in later years often referred to the many kindnesses he received from the professorial staff, notably Sir William Ramsay and Sir Flinders Petrie. He gained valuable experience there of the work of a large academic library and, in his turn, contributed not a little to improvements in procedure. It was no doubt the excellence of Clifford's work at the University College Library which led to his appointment in 1903 to the post which he was to fill with such success for the remaining 39 years of his life.

Up to 1919 the Library of the Chemical Society catered only for the Fellows and persons introduced by them, but in that year the Council of the Society offered usage of the Library to members of other Societies concerned with the progress of chemistry, in return for voluntary subscriptions towards the cost of maintenance. This offer was accepted by the Institute of Chemistry, the Society of Chemical Industry and other Societies, all of which were given representation on the Library Committee. This co-operation increased largely the number of potential readers and borrowers and, as the contributing societies were mainly concerned with applied chemistry, it was necessary to make the Library fully representative of all branches of the science and the purchasing policy of the Library Committee was modified accordingly. When, in due course, the Chemical Council was formed by agreement between the Chemical Society, the Institute of Chemistry and the Society of Chemical Industry, the present scheme of co-operation came into being. The Library remains the property of the Chemical Society, but its management is delegated to a Joint Library Committee on which the three organisations referred to above, and the contributing societies, have representation. This Committee is responsible to the Council of the Chemical Society for the purchase of books and to the Chemical Council for the cost of maintenance, that is, the cost of keeping the Library in working order. The Librarian has from the beginning acted as Secretary of the Committee. Clifford was a strong advocate of this co-operation and he and his staff spared no pains to make it a success in practice. One result has been a great increase in the library stock, which was about 15,000 volumes when Clifford became librarian in 1903 and is now about 45,000 volumes with, in normal times, an annual addition of nearly 1,000 volumes. The library premises proper still consist of two comparatively small rooms in which only a small fraction of the stock can be accommodated and which have also to serve as reading-room, office, packing-room, etc. The bulk of the library stock has to be stored in the basement and chemists owe much to Clifford's ingenuity in finding means of storing this huge accumulation of literature in the very limited space available and in keeping it more or less accessible. In spite of these difficulties, Clifford ran the Library with remarkable efficiency. He was, however, not only a good routine librarian. He knew the Library thoroughly and his knowledge of the literature of chemistry was probably unique. This knowledge he placed freely at the disposal of enquirers and his patience in searching for answers to knotty questions was phenomenal.

His competence as a librarian brought many requests for his services on committees and in other ways. For the British Cotton Industry Research Association in its early days he compiled a bibliography on cotton. He was a member of the Committee appointed by the British Science Guild to prepare the second and third editions of the Catalogue of British Scientific and Technical Books and he also served on the Advisory Committee responsible for the preparation of the World List of Scientific Periodicals. His knowledge of literature was not confined to the chemical variety, for he acted for some time as honorary librarian to the Arts Club and prepared a catalogue of the Club Library.

On the administrative side of library work he provided information for a report on public libraries in England and Wales issued by the Board of Education in 1927 and several of his suggestions were adopted in the report.

He always retained interest in municipal libraries and served on the Ealing Public Library Committee for many years and as Chairman from 1923 to 1927. His knowledge of bookbinding and of the materials of this craft led to his membership of the Bookbinding Leather Committee of the Printing Industry Research Association in 1932—1933.

He was a Fellow of the Library Association and for several years served on the Council of the Association of Special Libraries and Information Bureaux.

Clifford wrote comparatively little, but he did occasionally review books on bibliographical subjects and he published three papers for which references may be given, since they deal with the Library of this Society and are likely to be of special interest to readers of this journal (*Library World*, February, 1916, **18**, 228; *Library Association Record*, 1919, **21**, 234; *J. Soc. Chem. Ind.*, 1921, **40**, 424R).

It is probably not too much to say that Clifford's main object in life was the successful management of the Chemical Society's Library, but there was one other pursuit over which he was enthusiastic and to which he was probably led by his early experience of physical disability. At the age of 18 he obtained a First Aid Certificate. When war broke out in 1914 he was appointed Section Leader and later Quartermaster of the Middlesex Voluntary Aid Detachment and eventually was given a commission in the Royal Army Medical Corps (Vol.). After the war he recruited from his comrades the Ealing Division of the St. John Ambulance Brigade, of which he was made Superintendent in 1919 and promoted to District Officer in 1924. For his work in this sphere he was created a Serving Brother of the Order of St. John of Jerusalem in 1926 and an Officer of the Order in 1936. In 1942 he was given the rank of Commander in recognition of services rendered, especially in connection with the Library and Choir of the Order. He had a good singing voice and was a member of several choirs.

Clifford could not be described as a robust individual, but in his later years he had reasonably good health until the winter of 1939—40 when he had a serious illness. Early in 1942 he was again away from duty for several weeks, but he recovered and seemed to be as well as ever up to a few days before his unexpected death on Sept. 21st, 1942. He leaves a widow and one son.

Fellows of the Society, and indeed all who were accustomed to use the Society's Library, will long miss his genial presence and his unobtrusive and devoted service to the science we all profess. T. A. Henry.

## FRANK WILLIAM HARBORD.

### 1860—1942.

Frank William Harbord, who died on December 27th last in his 83rd year, was throughout his long life a prominent figure in the metallurgical world, and had been associated with important developments in the steel industry. He was born near Norwich in 1860 and educated at St. Olave's Grammar School, from which he entered the Royal School of Mines, gaining its Associateship in 1882. As metallurgical chemist in the works of Sir Alfred Hickman and of the Staffordshire Steel and Ingot Iron Co., and later as manager of the sheet works of Hatton, Sons and Co. at Bilston, he gained an intimate knowledge of the industry and especially of the basic Bessemer process, then in its early days. In 1892 he became metallurgical chemist to the Government of India, and until 1905 he was at the Royal Indian Engineering College at Cooper's Hill. In that year he took up private practice as a metallurgical analyst and consultant, and the firm of Riley and Harbord (later Riley, Harbord, and Law) has long held a prominent position in the metallurgical industries. Although his eyesight failed in later years, he retained his position as senior partner until his death. Much of his consulting work was concerned with railway engineering materials.

Although his published researches were few and confined to his earlier years, when he was much interested in the chemistry of the Bessemer and open-hearth steel-making processes, he contributed frequently to the discussions of the Iron and Steel Institute, and the question of the efficiency of steel works also engaged his attention. At the request of the Canadian Government he reported in 1904 on the position of electric smelting processes in Europe. During the war of 1914—18 he acted as honorary adviser in metallurgy to the Ministry of Munitions and was awarded the C.B.E. He was also an Officer of the Legion of Honour. His text book on the Metallurgy of Steel, of which he contributed the volume on metallurgy and Mr. John Hall that on the engineering aspects, passed through many editions and was for long the standard work in the English language. He took a keen interest in the work of the Iron and Steel Institute, of which he was President in 1927—28, and he was awarded the Bessemer Medal in 1916. He was a regular attendant at both the public and the Council meetings of the Institute, where his kindly character and his paternal interest in the younger metallurgists were well known. C. H. Desch.

## IVAN DOUGLAS LAMB.

### 1900—1942.

Ivan Douglas Lamb died on November 25th, 1942, after a short illness. He had been working in the laboratories until three weeks before his death and the news of his decease came as a shock to his colleagues and friends, who mourn the loss of a man of sterling character and high ability.

Lamb was born at Leicester on September 9th, 1900, and was educated at the Claremont School and at the Secondary School, Blackburn. After matriculation in the Northern Universities he went to the Manchester

Municipal College of Technology, where he graduated B.Sc. (Tech.) with Honours in 1922. Research work carried out under Prof. F. L. Pyman, F.R.S., on the chemistry of the glyoxalines led to the M.Sc. Tech. and to the publication of a paper in the *Journal*. After receiving his master's degree he was appointed, in 1923, to the scientific staff of the Wellcome Chemical Works, Messrs. Burroughs Wellcome and Co., Dartford. During the next two decades Lamb covered a wide field: he worked with equal facility on a diversity of subjects, but was particularly associated with technical work on the therapeutic compounds of arsenic and antimony, on glucosides, alkaloids and hormones. He will be specially remembered for his valuable work on the crystallisation of insulin on a technical scale. Towards the end of his career opportunities occurred for some essays in pure research and he carried out at the request of the British Pharmacopœia Commission a characteristically thorough study of the glucosides of *Strophanthus Emini*, part of which appeared in the *Journal*. Lamb also followed up the work of King, Laurie, and York by preparing a series of trypanocidal amidines and amidoximes, an account of which was published in the *Journal*. These activities were terminated by the war emergency and he again became immersed in technical problems.

Lamb was a keen angler and made long journeys in pursuit of his favourite sport. He was also fond of foreign travel and spent many of his holidays abroad. He was one of the earliest members of the Bexley A.R.P. Organisation, from which he transferred to the Home Guard, of which he became an enthusiastic and popular member.

S. Smith.

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## FRANK STURDY SINNATT.

**1880—1943.**

Born in Jersey in 1880, the son of Francis Sinnatt, Frank Sturdy Sinnatt moved to Manchester early in life. It was on this account that he was often heard to declare whimsically that he could never make up his mind whether he was a Jerseyman or a Mancunian. As his friends and associates well knew, however, this was, to him, a matter of no real importance, since his first allegiance, transcending all else, was to fuel technology.

He graduated in chemistry at the Manchester College of Technology. It was here, as Assistant Lecturer in chemistry, working under Sir William J. Pope on metallo-organic compounds, that he was drawn to the subject to which he devoted boundless energy to the end of his life. For many years he gave all his time to the study of coal in all its aspects—its winning from the earth, its preparation for the market, its use and treatment in industry, and its chemical and physical properties. All his vacations were spent at various works investigating the application of his subject to industry. Soon he was organising lectures on fuel and inspiring his students with enthusiasm for this new branch of technology, and it was not long before he was appointed Head of the Fuel Section in the College and Lecturer in Fuels in the University (1915). In this capacity he carried out and directed a variety of researches on coal and coal products which resulted in the publication of numerous scientific papers. During this period he still found the time and energy to join the Territorial Army in 1908. He went to France as a member of the Special Brigade, R.E., in the Great War, but to his great regret was forced to return to this country on medical grounds. Later he assumed command of the O.T.C. in the University.

Sinnatt was amongst the pioneers who realised the importance of carrying out a thoroughgoing survey of the nation's coal resources. In 1918 he participated in the formation of the Lancashire and Cheshire Coal Research Association, and became its first Director of Research. Within this Association were developed many of the methods of research which were to form the basis of the organisation of the Fuel Research Coal Survey. Sinnatt's part in this work will always be remembered as one of his major achievements.

In 1924 he was appointed Assistant Director of Fuel Research and Superintendent of the Coal Survey. In 1931 he became Director. His infectious enthusiasm and zest for his subject acted as a stimulus to every member of his staff, and the whole subject of fuel research received a powerful impetus. The Organisation was expanded; new avenues of research were opened up and pursued with vigour and success; the Coal Survey was firmly established and accepted as an essential adjunct to the mining industry; association with industry was fostered, and wherever possible results of research received practical application.

During all this time Sinnatt's devotion to his duties in the Fuel Research Organisation did not deter him from playing a lively part in other scientific activities. This is witnessed by his service on the councils of the Institution of Mining Engineers, the Institute of Fuel, the British Iron and Steel Federation, the British Colliery Owners Research Association, The British Coal Utilisation Research Association and on the British National Committee and Executive Committee of the World Power Conference. In addition he was a member of the Coke Oven Managers' Association, the Institution of Gas Engineers and the Royal Swedish Institute for Engineering Research and he was an original member of the Coal Research Club.

His work in the field of fuel technology did not pass without recognition. Manchester University conferred the degree of D.Sc. upon him, and in 1938 he received the highest honour the scientific world can bestow by his election to the Fellowship of the Royal Society. He was awarded the M.B.E. (Mil.) for his services during the Great War, and later he received the C.B. (1935).

Since the outbreak of war he gave himself unstintingly to many branches of scientific research and technical developments for the furtherance of the war effort. His death has robbed the nation of one of its foremost technologists.

He was elected a Fellow in February, 1902.

J. G. King.

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## 49. *The Stereochemical Relationships of the* 9 : 10-*Oxidostearic Acids and the* 9 : 10-*Dihydroxystearic Acids.*

By D. Atherton and T. P. Hilditch.

When either of the two isomeric forms of 9 : 10-oxidostearic acid is treated with an ethereal solution of hydrogen chloride, the oxido-ring is opened and chlorohydroxystearic acids are produced. The latter in presence of alkali undergo ring closure and the oxidostearic acid thus re-formed is identical with that from which the experiment commenced. It follows that the inversion, which occurs when either of the 9 : 10-dihydroxystearic acids is converted into chlorohydroxy-acids and the latter, through the oxido-compounds, into the isomeric form of the 9 : 10-dihydroxystearic acid, must take place during replacement of a hydroxyl group by chlorine.

This leads to the conclusion that no inversion takes place during the conversion of oleic and elaidic acid into the 9 : 10-dihydroxystearic acids melting at 95° and 132° respectively by means of perbenzoic, peracetic, or Caro's acid.

It has recently been demonstrated (King, J., 1942, 387) that either form of the 9 : 10-dihydroxystearic acids, m. p. 132° or 95°, when heated with hydrogen chloride at 160°, yields chlorohydrins, from which oxidostearic acids may be obtained which, on opening of the oxide ring, yield the opposite form of the dihydroxy-acids (m. p. 95° or 132°). Following Böeseken and Belinfante (*Rec. Trav. chim.*, 1926, **45**, 917) and Esafov (*J. Gen. Chem. Russia*, 1937, **7**, 1403), who have suggested that inversion always occurs when α-oxides are hydrated, King has formulated the stereochemical course of these transformations on the assumption that inversion occurs only during the opening of the oxide ring. This conclusion harmonises well with the low optical activity of the naturally-occurring 9 : 10-dihydroxystearic acid, m. p. 141°, which, since it yields the acid of m. p. 95° when submitted to the above sequence of reactions, must be an optically active form of the racemic acid of m. p. 132°; and, according to King's scheme, the latter acid would be a racemic mixture of enantiomorphs in which the rotations due to each asymmetric centre would have opposing influences.

We have sought for further experimental evidence bearing on this question and have ascertained the following facts:

(i) The oxidostearic acid obtained from the 9 : 10-dihydroxystearic acid, m. p. 95°, by King's procedure, that obtained from the chlorohydroxystearic acid produced by addition of hypochlorous acid to elaidic acid, and that obtained by perbenzoic acid oxidation of elaidic acid, are all identical.

(ii) The oxidostearic acids correspondingly obtained from the isomeric dihydroxystearic acid of m. p. 132°, and from the chlorohydroxystearic acid produced by addition of hypochlorous acid to oleic acid, are also identical with each other, but differ from those referred to under (i).

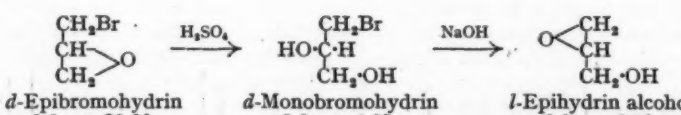
In our experience the melting points of both forms of the 9 : 10-oxidostearic acid are practically the same, namely, 55—56° in cases (i) and 56—57° in cases (ii); but mixture of either of the specimens (i) with either of the specimens (ii) produced a depression in melting point of 8—10°. It was also noticeable that the oxidostearic acid (ii) was much more soluble in organic solvents than the oxidostearic acid (i), this behaviour being parallel with the relative solubilities of the dihydroxystearic acids of m. p. 95° and 132°.

(iii) The oxide ring of both forms of the oxidostearic acids has been opened by treatment with an ethereal solution of hydrogen chloride, leading to the production of chlorohydroxystearic acids which were subsequently reconverted into oxidostearic acids. The oxidostearic acid recovered was in each case the same isomeric form as that originally employed. Thus, an oxidostearic acid (m. p. 55—56°) prepared from the product of addition of hypochlorous acid to elaidic acid yielded a chlorohydroxy-acid [Found: Cl, 9·3; OH (non-carboxylic), 4·6. $C_{18}H_{35}O_3Cl$ requires Cl, 10·6; OH (non-carboxylic), 5·1%]. This chlorohydroxy-acid gave on ring-closure an oxidostearic acid which melted at 54—55° [mixed with the original oxidostearic acid, m. p. 54—56°; mixed with the isomeric oxidostearic acid (ii), m. p. 44—46°].

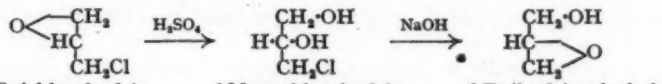
These experiments prove either that there is no inversion during either opening or closing of the oxide ring, or that inversion takes place during both these processes. It is not necessary, for the immediate purpose of our argument, to determine which of these alternatives is operative; nevertheless the following points may be mentioned which seem to suggest the absence of a Walden inversion at either stage:

(*a*) Hydrolysis of the oxido-system by alkali or by sulphuric acid (Steger and van Loon, *Rec. Trav. chim.*, 1927, **46**, 702) leads to formation of the same isomeric form of dihydroxystearic acid. Since the mechanism of ring fission in an acidic medium probably includes intermediate formation of an oxonium salt complex, it seems improbable that in this case, or in the corresponding production of a chlorohydrin from the oxido-compound by hydrogen chloride in ether, any inversion should occur.

(*b*) Moreover, Abderhalden (*Ber.*, 1915, **48**, 1847) studied the conversion of optically active epihalogenohydrins by sulphuric acid into monohalogenohydrins, and of the latter by alcoholic alkali into epihydrin alcohols with the following results:




*d*-Epibromohydrin $[\alpha]_D + 23{\cdot}1°$. *d*-Monobromohydrin $[\alpha]_D + 4{\cdot}8°$. *l*-Epihydrin alcohol $[\alpha]_D - 8{\cdot}5°$.




*l*-Epichlorohydrin $[\alpha]_D$ − 25·6°. *l*-Monochlorohydrin $[\alpha]_D$ − 1·9°. *d*-Epihydrin alcohol $[\alpha]_D$ + 7·7°.

Although in these sequences of changes the optical rotation changes in sign owing (as can be seen more clearly from three-dimensional models) to the opening of the oxido-ring on the α and β carbon atoms and subsequent production of a new oxido-ring from the β and α′ carbon atoms of the three-carbon chain, no Walden inversion has taken place, *i.e.*, there has been no spatial rearrangement of the groups attached to the middle asymmetric carbon atom.

Whether the two-fold inversions occur or not, it follows from the experiments described under (iii) above that the chlorohydrins obtained from dihydroxystearic acid have the same configuration as the dihydroxystearic acids produced therefrom through the oxidostearic acids, and therefore that the first stage of the conversion of one form of 9 : 10-dihydroxystearic acid into the other—the formation of " chlorohydrins " by the action of hydrogen chloride at 160°—must be accompanied by a Walden inversion. This, indeed, is in harmony with the views at present accepted in regard to the substitution of one anionic group for another (cf. Watson, *Ann. Reports*, 1938, **35**, 222; Kenyon and Phillips, *Trans. Faraday Soc.*, 1930, **26**, 451; Olson, *J. Chem. Physics*, 1933, **1**, 418; Meer and Polanyi, *Z. physikal. Chem.*, 1932, *B*, **19**, 164).

This proof that inversion occurs during replacement of one of the hydroxyl groups of dihydroxystearic acid by chlorine leads to the further implications that no change in stereochemical configuration takes place during addition of hypochlorous acid to oleic or elaidic acid or during the oxidation of these acids by perbenzoic, peracetic or Caro's acid. We have made several attempts to detect the formation of oxidostearic acid in the course of the last two oxidations, but without success.* A possible explanation of the production of oxido-

*Diagram* 1.

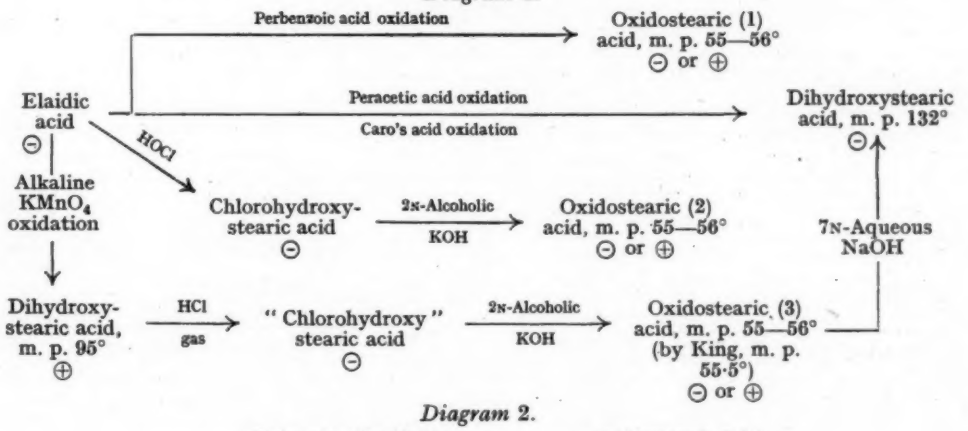
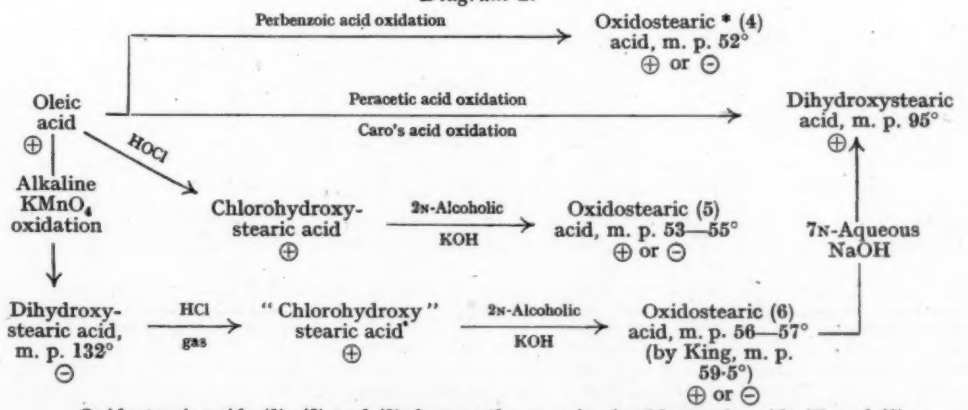


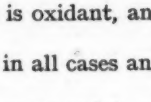

*Diagram* 2.




Oxidostearic acids (1), (2) and (3) depress the m. p.'s of oxidostearic acids (5) and (6). Mixtures of (1) and (2) and (3), or (5) and (6), melt without depression.

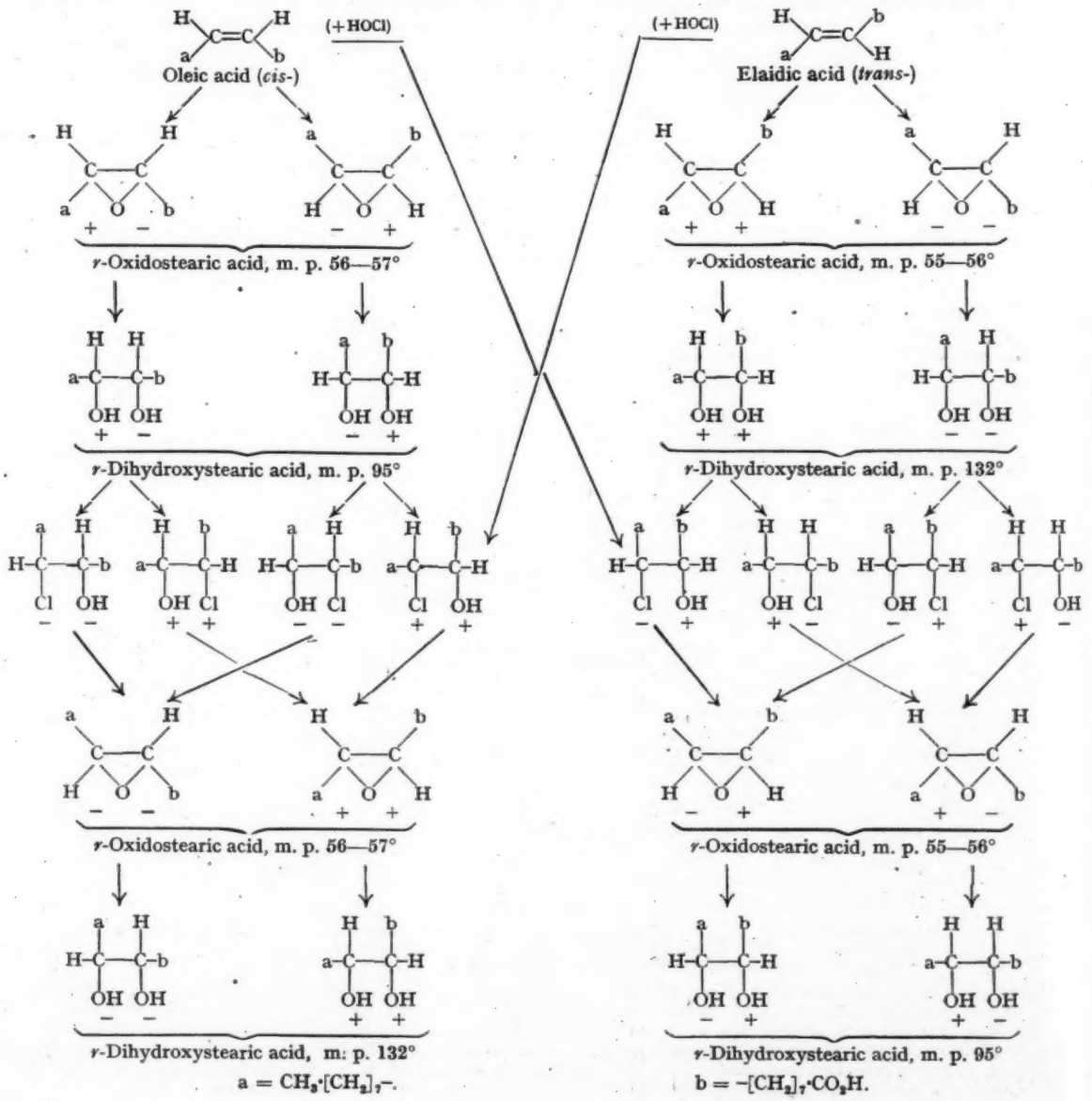
* Böeseken and Belinfante, *Rec. Trav. chim.*, 1926, **45**, 917.

* Since this paper was submitted, King (this vol., p. 37) has shown that, under restrained conditions of oxidation with hydrogen peroxide and acetic acid, elaidic acid furnishes the oxidostearic acid, m. p. 55·5°, in 25—30% yield. He has also observed that both oxidostearic acids, on prolonged standing with cold acetic acid, are converted into monoacetyl derivatives of the corresponding dihydroxystearic acids. In presence of acetic acid, oleic acid epoxide undergoes ring fission, to the monoacetyl derivative of the dihydroxystearic acid, m. p. 95°, much more readily than elaidic acid epoxide, which is partly resolved into the monoacetyl derivative of the dihydroxystearic acid, m. p. 132°.

stearic acids when perbenzoic acid is oxidant, and of dihydroxystearic acids exclusively when peracetic acid or Caro's acid is used, may be that in all cases an oxonium addition-compound  is formed with the peracid but in the latter two instances (in which, moreover, the action proceeds in an acid solution) the presence of the stronger acid—sulphuric or acetic—causes instantaneous opening of the oxide ring, which, however, resists rupture in the less strongly acidic conditions of oxidation by perbenzoic acid in a neutral solvent.

We believe that, in the light of the present results, the changes operating in these various actions are accurately summarised in diagrams 1 and 2 (p. 205), in which the signs (+) and (−) are arbitrarily given to oleic and elaidic acid in order to correlate the configurational relationships (the signs of course have no reference to optical rotations). To indicate the uncertainty as to whether inversion occurs during closure and fission of the oxide ring, or in neither case, the oxidostearic acids are given both (+) and (−) signs.

It will be observed that, according to this scheme, the production of dihydroxystearic acid by alkaline permanganate oxidation of the ethenoid acid must involve an inversion. It has been suggested (Hilditch, J., 1926, 1828; Hilditch and Lea, J., 1928, 1576) that this is connected in some way with the marked excess of alkali which Lapworth and Mottram (J., 1925, 127, 1628) showed to be essential for the production of good yields of dihydroxystearic acid by this procedure. Even with excess of alkali as recommended by Lapworth and Mottram, the stable *trans*-isomeride elaidic acid affords definitely lower yields of the dihydroxystearic acid,


(+HOCl)
Oleic acid (cis-)
(+HOCl)
Elaidic acid (trans-)
γ-Oxidostearic acid, m. p. 56—57°
γ-Oxidostearic acid, m. p. 55—56°
γ-Dihydroxystearic acid, m. p. 95°
γ-Dihydroxystearic acid, m. p. 132°
γ-Oxidostearic acid, m. p. 56—57°
γ-Oxidostearic acid, m. p. 55—56°
γ-Dihydroxystearic acid, m. p. 132°
γ-Dihydroxystearic acid, m. p. 95°

a = $CH_3 \cdot [CH_2]_7 -$.

b = $-[CH_2]_7 \cdot CO_2H$.

m. p. 132°, than the almost quantitative yields of the acid, m. p. 95°, obtainable from oleic acid by aqueous alkaline permanganate (Saytzew, *J. pr. Chem.*, 1886, **34**, 315; Hilditch, *loc. cit.*). These observations support the view that the alkalinity of the medium determines the configurational course taken during the conversion of the double bond in monoethenoid higher aliphatic acids into a glycol by oxidation.

As pointed out by King (*loc. cit.*), occurrence of inversion during replacement of hydroxyl by chlorine, instead of solely at the stage when oxidostearic acid is converted into dihydroxystearic acid, would involve different configurational formulæ for the products produced in the later stages of his reaction from those which he formulated (*loc. cit.*, p. 388). According to our present results, this scheme takes the form shown on p. 206 (where an inversion during replacement of hydroxyl by chlorine is allowed for, and closure and opening of the oxide ring are assumed to take place without inversion).

According to this scheme the active 9 : 10-dihydroxystearic acid, m. p. 141°, which King has shown to be one of the components of the racemic acid of m. p. 132° would be a diastereoisomeride of the form *d*A*d*B and might therefore be expected to exhibit stronger rotatory power than has been observed. Although this feature is apparently in conflict with the rest of the evidence discussed above, it may be pointed out that there is little knowledge available whereby the probable order of rotatory power of such a compound might be predicted. Further work on the resolution of both racemic dihydroxystearic acids, m. p. 132° and 95°, on which data hitherto recorded are not in agreement (Freundler, *Bull. Soc. chim.*, 1895, **13**, 1053; Inoue and Suzuki, *Proc. Imp. Acad. Tokyo*, 1931, **7**, 261; Toyama and Ishikawa, *Bull. Chem. Soc. Japan*, 1936, **11**, 735), appears to be necessary before this point can be settled.

## Experimental.

*Interconversion of the* 9 : 10-*Dihydroxystearic Acids.*—The results obtained by King (*loc. cit.*) were substantially reproduced, except that, as already mentioned, the m. p.'s of the oxidostearic acids differed somewhat from those recorded by King. Further, the products of the action of hydrogen chloride at 160° on the dihydroxystearic acids were found by analysis to consist not of chlorohydroxystearic acids, but almost wholly of chloro-esters, or, rather, chloro-estolides, one of the hydroxyl groups of the dihydroxystearic acid having been esterified, under the conditions employed, with a carboxyl group (the latter probably from a different molecule of the acid).

9 : 10-Dihydroxystearic acid, m. p. 132° (5 g.), was heated for 4 hours at 160° in an open tube in a current of dry hydrogen chloride. The product, after solution in ether, washing with water and recovery from solvent, was a brown viscous oil (4·8 g.) [Found : Cl, 10·1; OH (carboxylic), 0·6; OH (non-carboxylic), 0·6%. $C_{18}H_{33}OCl$ requires Cl, 11·2%; OH, nil %].

The chloro-estolides (4·1 g.) were refluxed for 2 hours with 2N-alcoholic potassium hydroxide (50 c.c.) and yielded 3·8 g. of solid acids which, on crystallisation, gave 0·7 g. of impure dihydroxystearic acid, m. p. 87°, and 2 g. of oxidostearic acid (No. 6, diagram 2), which after repeated crystallisation from methyl alcohol and acetone melted at 55—57°. This oxidostearic acid yielded the dihydroxystearic acid, m. p. 93—94°, after heating with excess of 7N-aqueous sodium hydroxide in a closed tube at 170°.

9 : 10-Dihydroxystearic acid, m. p. 95° (5·2 g.), was similarly treated with hydrogen chloride at 160° and yielded a dark brown oil (5·1 g.) [Found : Cl, 10·6; OH (carboxylic), 0·5; OH (non-carboxylic), 0·6%]. The oil (4·6 g.) was converted by refluxing with 2N-alcoholic potassium hydroxide into solid acids, which after repeated crystallisation gave oxidostearic acid (No. 3, diagram 1), m. p. 55—56° [depressed to 46—47° on admixture with the preceding oxidostearic acid (No. 6), unchanged when mixed with oxidostearic acid (No. 1) from perbenzoic acid oxidation of elaidic acid]. The oxidostearic acid No. 3 was much less soluble in light petroleum, methyl alcohol and acetone than the acid No. 6.

*Chlorohydroxystearic Acids from Oleic and Elaidic Acids.*—These were prepared by dissolving the fatty acid (20 g.) in water (200 c.c.) containing sodium hydroxide (3 g.) and mechanically stirring the solution, through which carbon dioxide was passed during the slow addition of sodium hypochlorite solution (70 c.c. containing 9% of NaOCl). The resulting solution was made acid with dilute sulphuric acid, and the product recovered by extraction with ether.

Elaidic acid thus yielded a semi-solid mixture of chlorohydroxystearic acids [Found : I.V. (Wijs), 8·3; Cl, 9·8; OH (non-carboxylic), 3·5. $C_{18}H_{35}O_3Cl$ requires Cl, 10·6; OH (non-carboxylic), 5·1%]. On crystallisation of the semi-solid acids a small amount of a solid chlorohydroxystearic acid was obtained, m. p. 58°. The semi-solid chlorohydroxystearic acids (8 g.) gave, on refluxing with 2N-alcoholic potassium hydroxide (100 c.c.), a solid product (7·2 g.) which on crystallisation gave 0·3 g. of impure dihydroxystearic acid, m. p. 132°, and 3·6 g. of oxidostearic acid (No. 2, diagram 1) which after repeated crystallisation melted at 55—56° (unchanged when mixed with oxido-acids No. 1 and No. 3, depressed to 43—46° when mixed with oxido-acid No. 5, diagram 2).

Oleic acid, which reacted more completely than elaidic acid with hypochlorous acid, gave a solid product [Found : I.V. (Wijs), 1·4; Cl, 10·7; OH (non-carboxylic), 4·0%]. The latter (8 g.), refluxed with 2N-alcoholic potassium hydroxide (50 c.c.), gave a viscous oil (7·7 g.) which solidified after standing in an exsiccator for 2 days. It was crystallised only with difficulty and furnished 0·5 g. of impure dihydroxystearic acid, m. p. 95°, and 0·6 g. of oxidostearic acid (No. 5, diagram 2), m. p. 54° (unchanged when mixed with oxido-acid No. 6, depressed to 48—52° when mixed with oxido-acid No. 1).

The yield of oxido-acid was much improved by first converting the chlorohydroxy-acids into chloro-estolides. The chlorohydroxystearic acids (16·0 g.) from oleic acid were heated at 160—170° for some hours in presence of a trace of camphor-$\beta$-sulphonic acid and in an atmosphere of carbon dioxide. The product (15·4 g.) was a dark viscous oil [Found : Cl, 9·9; OH (non-carboxylic), 0·7%], 14·7 g. of which, on refluxing with 2N-alcoholic potassium hydroxide, gave a semi-solid product (12·8 g.), from which 0·4 g. of impure dihydroxystearic acid, m. p. 91·5—93°, and 6·4 g. of crude oxidostearic acid were isolated. The latter, on repeated crystallisation gave 2 g. of oxidostearic acid, m. p. 52—53° (mixed with the preceding oxido-acid No. 5, m. p. 52—54°).

*Opening and Re-closing of the Oxide Ring in the Oxidostearic Acids.*—The oxidostearic acid No. 2 (from elaidic acid through chlorohydroxystearic acid) (3·1 g.) was dissolved in a saturated solution of dry hydrogen chloride in ether (100 c.c.) and kept for 18 hours; it was then refluxed for 1 hour with 10 c.c. of water (Nicolet and Poulter, *J. Amer. Chem. Soc.*, 1930, **52**, 1180). The solid product (3·4 g.) [Found : Cl, 9·3; OH (non-carboxylic), 4·6%] gave on repeated crystallisation from light petroleum a small amount of acid, m. p. 64—65° [mixed with the original chlorohydroxy-acid (m. p. 58°), m. p. 59—62°].

The chlorohydroxystearic acid (2·7 g.) thus produced, refluxed with 2N-alcoholic potassium hydroxide for 4 hours, was converted into crude oxidostearic acid which, on crystallisation, yielded 0·1 g. of impure dihydroxystearic acid,

m. p. 132°, and 0·4 g. of oxidostearic acid, m. p. 54—55° [mixed with the original oxidostearic acid No. 2 used in the experiment, m. p. 54—56°; mixed with oxidostearic acid No. 5 (from the chlorohydroxy-acid from oleic acid), m. p. 44—46°].

Similarly, the oxidostearic acid No. 5 (from oleic acid through chlorohydroxystearic acid) (3·1 g.) was converted by dry hydrogen chloride in ether into semi-solid chlorohydroxy-acids [Found: Cl, 8·0; OH (non-carboxylic), 5·3%], which, when refluxed with 2N-alcoholic alkali gave, after crystallisation of the solid product, 0·2 g. of dihydroxystearic acid, m. p. 95°, and 1·2 g. of crude oxidostearic acid which after repeated crystallisation melted at 52—54° [mixed with the original oxidostearic acid No. 5 used in this experiment, m. p. 52—53°; mixed with oxidostearic acid No. 2 (from the chlorohydroxy-acid from elaidic acid), m. p. 40—46°].

*Oxidation of Elaidic Acid with Perbenzoic Acid.*—Elaidic acid (6·8 g.) was oxidised in chloroform solution (15 c.c.) with perbenzoic acid as described by Böeseken (*Rec. Trav. chim.*, 1926, **45**, 917). From the product (8·2 g.) there were obtained by crystallisation 0·3 g. of dihydroxystearic acid, m. p. 130°, and 1·6 g. of oxidostearic acid (No. 1, diagram 1), m. p. 55°. The methyl-alcoholic mother-liquors from the oxidostearic acid, on hydrolysis in the cold with N/2-alcoholic potassium hydroxide, gave a large yield of dihydroxystearic acid, m. p. 132°.

*Oxidation of Elaidic Acid with Peracetic Acid.*—Elaidic acid, when oxidised with perhydrol in glacial acetic acid (Hilditch, *loc. cit.*; Scanlan and Swern, *J. Amer. Chem. Soc.*, 1940, **62**, 2304), gave the dihydroxystearic acid, m. p. 132°, in almost theoretical yield and no evidence of the presence of oxidostearic acid could be detected.

A partial oxidation was then carried out in the following manner in the hope of isolating the oxido-acid; elaidic acid (5 g.) was refluxed for 3 hours in acetone solution (50 c.c.) with an excess of peracetic acid (4 c.c. of 30% hydrogen peroxide with 4·25 c.c. of glacial acetic acid). The product (5·5 g.) gave on crystallisation 0·54 g. of dihydroxystearic acid, m. p. 126—128°, and 0·9 g. of unchanged elaidic acid. The soluble portion was refluxed with N/2-alcoholic potassium hydroxide (50 c.c.) in order to hydrolyse any acetylated products; the recovered acids gave a further 0·4 g. of dihydroxystearic acid, m. p. 128°, and 2 g. of an oily residue (I.V. 47·5). No oxidostearic acid was detected.

*Partial Oxidation of Elaidic Acid with Caro's Acid.*—Elaidic acid (5 g.) was dissolved in acetone (50 c.c.) containing 1·95 c.c. of sulphuric acid and 2·1 c.c. of 30% hydrogen peroxide. No reaction set in in the cold and the solution was refluxed for 1 hour and the acids were then recovered. On crystallisation a very small amount (0·1 g.) of the dihydroxystearic acid, m. p. 132°, was obtained together with unchanged elaidic acid, but no oxidostearic acid could be detected.

We are indebted to I.C.I. (Dyestuffs) Ltd. for permission to publish these results.

THE UNIVERSITY, LIVERPOOL. [*Received, January* 12*th*, 1943.]

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## 50. *Physicochemical Aspects of Bacterial Growth. Part VIII. Growth of* Bact. Lactis Aerogenes *in Media containing Ammonium Sulphate or Various Amino-acids.*

By R. M. LODGE and C. N. HINSHELWOOD.

When *Bact. lactis aerogenes* is grown in a liquid synthetic medium containing ammonium sulphate or various amino-acids as the sole source of nitrogen: (*a*) The maximum population of cells which the medium supports varies very widely with the nitrogen source. (*b*) For certain nitrogen sources this population is greatly increased by aeration of the solution; for others not. (*c*) In contrast with this diversity, the growth rates all fall within a comparatively narrow range. (*d*) After a slight mutation had occurred, the growth rate in the ammonium sulphate medium increased by about 40% and the maximum population now showed a positive response to aeration, previously absent. (With certain amino-acids there was no such change in behaviour.)

It is suggested that the maximum population is in part controlled by the formation of an inhibiting substance removable by a cellular oxidation; that the earliest steps in the utilisation of the amino-acids are not usually rate-determining; and that the mutation involved a slight change in a delicate balance of these and other reactions. Similar considerations help to explain occasional departures from logarithmic form of the growth curve.

THE different phases of bacterial growth, namely, lag, logarithmic and stationary phases, are separately influenced by such factors as concentrations of medium constituents, pH, and the presence of foreign substances (Parts IV, V, and VI; J., 1939, 1683, 1692; 1940, 1565). It is to be hoped that the detailed investigation of these effects will assist in the analysis of some of the physicochemical mechanisms underlying cell growth.

In the experiments to be described the nitrogen source in the growth medium was varied. *Bact. lactis aerogenes* was grown in a solution containing glucose (38·5 g./l.), potassium dihydrogen phosphate (3·46 g./l.), brought to pH 7·12 with sodium hydroxide, and magnesium sulphate (0·038 g./l.) together with various nitrogen sources, ammonium sulphate (0·96 g./l.) or different amino-acids giving an equivalent supply of nitrogen. The culture was that used in previous work: it was usually subcultured, just before use, in the artificial medium, from which it was inoculated into the culture medium by a calibrated pipette of about 0·1 c.c. Cell counts were made in a hæmocytometer as previously described. The number ($n$) tabulated below is the number in 16 small squares of the counting grid: multiplied by $1{\cdot}25 \times 10^6$ it gives the number per c.c.

In the first series of experiments the artificial medium was inoculated with two or three loops of a bouillon culture and incubated overnight. Next morning 0·1 c.c. of the young culture, which was counted, was transferred to a tube of new medium. The mean generation time (*m.g.t.*) and the steady population attained at 24 hours ($n_{24}$) were determined; $n_0$, the initial count, was known from the count of the inoculum and the ratio of volumes of inoculation pipette and growth medium. The curves of the logarithm of the count, $n$, against time usually showed a well-defined straight portion from which the *m.g.t.* could be calculated. By extrapolating to the time where $n = n_0$, the length of the lag phase could be read off.

The results may be grouped as follows.

(1) Under the conditions of these experiments the lag was nearly zero with different amino-acids, but with ammonium sulphate it was variable and sometimes considerable. This fact is illustrated in Fig. 2, in which

$\log n/n_0$ is plotted against time; where the line passes through the origin the lag is zero; otherwise the intercept on the time axis where $\log n/n_0 = 0$ gives the lag. The whole question of lag is dealt with more fully in the next paper.

(2) The maximum population of cells in media containing different amino-acids varies widely. With certain amino-acids, though not with others, it also depends in the most marked way upon whether or not a current of air (normal carbon dioxide content) is passed through the medium (Table I). As shown in Part IV (*loc. cit.*),

TABLE I.

| Nitrogen source. | $n_{24}$. Solutions aerated. | Solutions not aerated. |
|---|---|---|
| Glycine | 34, 63 (110) * | 122 |
| Alanine | 1470, 1040, 1390, 815 (2500) | 235, 40, 125 |
| α-Amino-*n*-butyric acid | 20 | 11 |
| ,, ,, ,, | 790 | 72 |
| α-Amino*iso*butyric acid | 23, 22 | 18, 16 |
| α-Amino-*n*-valeric acid | 300 | 230 |
| Valine | 1230, 1205 (1440) | 55 |
| Leucine | 740, 740 (740) | 72 |
| Cysteine | 46, 48 | 50, 53 |
| α-Amino-octoic acid | 124 | — |
| Aspartic acid | 2100, 2240 | 630 |
| Glutamic acid | 1570, 1550 (2900) | 50 |
| Asparagine | 4800 | 1000 |
| Ammonium sulphate | 560 (2140, 1780) | 530 (470, 530) |
| ,, ,, | 550 | 540 |
| $(NH_4)_2SO_4$ + glycine | — | 485 |
| $(NH_4)_2SO_4$ + valine | — | 380 |

TABLE II.

*Mean generation times.*

(The solutions were aerated but in the early stages of growth aeration made little difference to the *m.g.t.*)

| Nitrogen source. | *m.g.t.* (mins.). | Nitrogen source. | *m.g.t.* (mins.). |
|---|---|---|---|
| Ammonium sulphate | 46 (32) | Aspartic acid | 39 |
| Glycine | 49 | Glutamic acid | 40 |
| Alanine | 41 (40) | Asparagine | 30·5 (29·2) |
| Valine | Early 40 (—) | Leucine | 36 |
| ,, | Late 100 (114) | α-Amino-octoic acid | <42 |

* The values in parentheses were measured after the slight mutation of the bacterium.

the setting in of the stationary phase is determined, according to conditions, either by the exhaustion of food or by the accumulation of substances adverse to growth (including adverse pH). The early setting in of the stationary phase with some of the unaerated amino-acids is not due to exhaustion or to wastage of nitrogen in side reactions: this is proved by the following experiment. $n_{24}$ for aerated alanine was 1470, for unaerated alanine only 125. On subsequent aeration of the latter the count rose to 1390. The cessation of growth in the first instance was therefore probably due to the accumulation of an inhibitor removable by aeration. This hypothesis can reasonably be extended to explain the early cessation of growth with some of the other amino-acids even in presence of air: here we have only to assume that the inhibiting product is not capable of sufficiently rapid removal by oxygen.

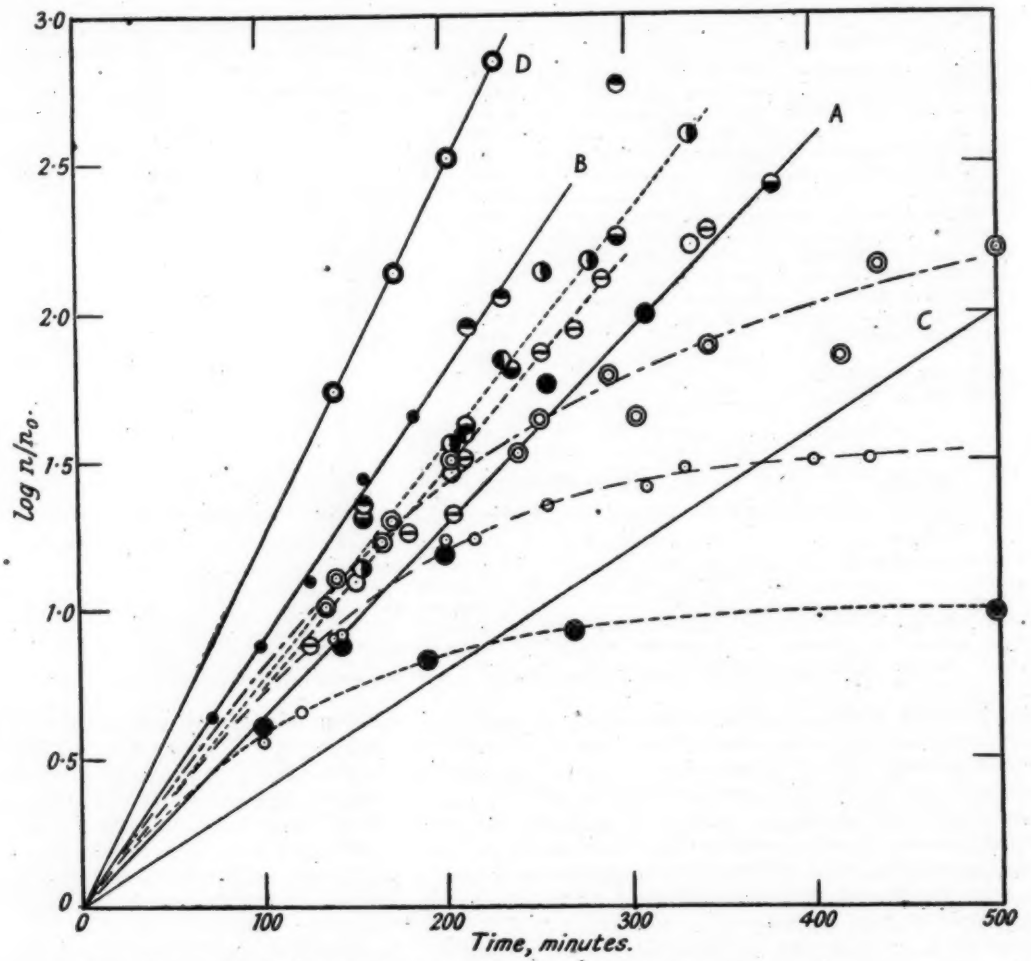
The removal is probably an oxidation and not the blowing off of a volatile product, since growth rates and stationary populations are practically independent of the actual rate of aeration. When two nitrogen sources are present together, one giving by itself a high value of $n_{24}$ and the other a low, the actual value is close to the higher. For example, ammonium sulphate unaerated gave $n_{24} = 535$, glycine and valine gave respectively 122 and 55. Ammonium sulphate in presence of the two respective amino-acids gave 485 and 380. Either the ammonium sulphate inhibits toxin formation from the amino-acids, or else the substances which they produce and which would inhibit their own utilisation fail to affect the processes by which ammonium sulphate is utilised in the cell. A similar effect in which amino-acids which support a small $n_{24}$ fail to inhibit growth to large $n_{24}$ values in others is illustrated in Fig. 2.

One might have supposed that in all these experiments the limiting factor was oxygen supply, but this hypothesis would not easily explain the very great differences between the various amino-acids, and the fact that, with some, growth responds to aeration, and with others not.

It might have appeared profitable to try correlating the above-mentioned differences with the structure of the amino-acids. The following observations seem, however, to show that they depend upon a very delicate quantitative balance of the enzyme processes of the cell. Some months after the first experiments were made the bacteria underwent a slight mutation—not detectable by any qualitative criterion and only shown in slight changes in growth rate (see later). With ammonium sulphate, aeration, which had previously made little difference to $n_{24}$, now had a marked effect, the values of $n_{24}$ for the unaerated and the aerated cultures being on the average 500 and 2000 respectively. In some of the other cases there was little change.

(3) Although the values of $n_{24}$ varied widely for the different nitrogen sources, the mean generation times all fell within a comparatively restricted range. This is shown in Fig. 1 and Table II. In the early experiments ammonium sulphate gave a *m.g.t.* of 45′ and the fastest growth occurred in asparagine where the *m.g.t.* was 29′. As seen in Fig. 1, change of the amino-acid affected the *m.g.t.* far less than a change to another medium such as bouillon, lactose–phosphate or lactose–tartrate. Where the value of $n_{24}$ is small the *m.g.t.* is hard to determine, but from the composite plot in Fig. 1 it looks probable that the initial growth rates with very many

FIG. 1.




- Ammonium sulphate (slow type). Line OA.
- dl-Valine (two curves).
- Meat extract ('heart broth'). Line OD.
- α-Aminoisobutyric acid.
- l-Leucine.
- Asparagine.
- Aspartic acid.
- dl-α-Aminooctoic acid.
- dl-Alanine (two curves).
- Glutamic acid.
- Glycine.
- Ammonium sulphate (fast type) Line OB.

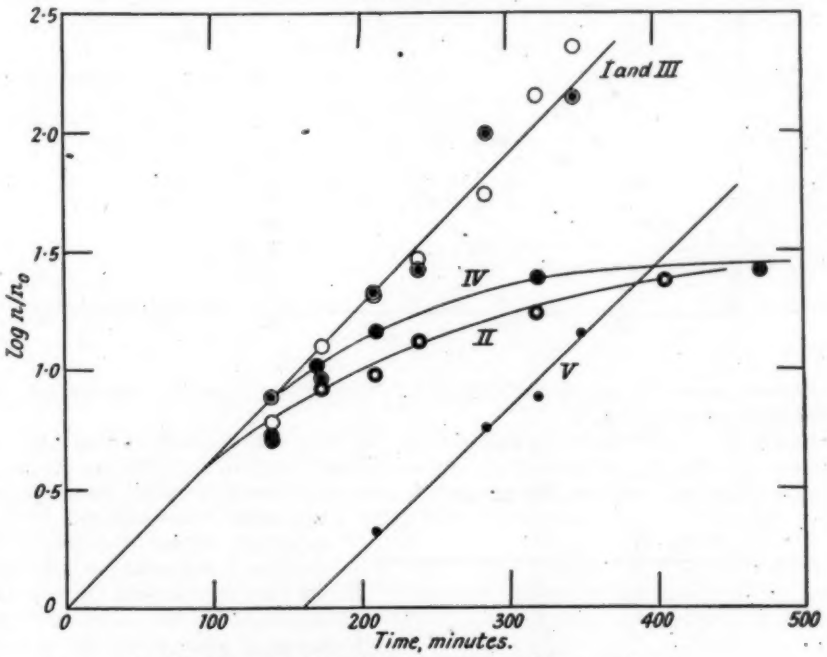
of the amino-acids are really very closely similar even where the *m.g.t.* is not accurately tabulated in Table II. Mixtures of several amino-acids gave no particular advantage, as is shown by the curves in Fig. 2.

(4) After sub-culturing for about a year, the *Bact. lactis* was observed to have undergone a slight mutation. (Plating out and application of qualitative tests showed that no infection had occurred.) The $n_{24}$ values for several amino-acids in the glucose–phosphate medium were unchanged (Table I) but there was now a marked influence of aeration upon growth in the ammonium sulphate medium. Aeration now increased $n_{24}$ (see Table I), and, whereas formerly aerated and unaerated cultures had both shown an *m.g.t.* of 45′, aerated growth was

now speeded up (average *m.g.t.* = 32′), while unaerated growth was slower (*m.g.t.* 40′ or longer). The *m.g.t.* with alanine (aerated) was 40′, *i.e.*, not much change; two determinations with glutamic acid gave 46′ and 46′ (again little change), whereas leucine gave considerably slower growth. The growth curve originally obtained with valine was non-logarithmic (Fig. 1). Initially the *m.g.t.* was about 40′ but this lengthened and over a considerable range before the stationary phase set in it remained constant at about 100′. After the mutation, values found were 114′ and 112′. In Fig. 1 the lines *OA* and *OB* represent the growth rates in ammonium sulphate before and after the mutation. It is to be noted that practically all the rates in different amino-acids lie *within* this range. *OC* refers to growth in a lactose–phosphate–ammonium sulphate medium.

(5) Usually the growth curve was of well-defined logarithmic form from the end of the lag phase nearly to the point at which the rapid turn over to the stationary phase occurred [Fig. 3 (*a*), (*b*)]. There were, however, occasional exceptions, significant enough to be worth noting and discussing. (i) A *gradual* transition from the lag to the true logarithmc phase. This effect was specially in evidence with ammonium sulphate after the mutation. It is illustrated in Fig. 3 (*d*). (ii) An arrest in growth for a time [Fig. 3 (*e*)], after which it is

FIG. 2.




I. *Aspartic acid, glutamic acid, and asparagine.*
II. *Glycine, α-aminoisobutyric acid, and α-amino-octoic acid.*
III. *All six of the above.*
IV. *Glycine with traces of tyrosine and tryptophan.*
V. *Ammonium sulphate (showing lag).*

*(All solutions unaerated.)*

resumed, sometimes even making up lost time, as in Fig. 3 (*c*). (iii) Growth slows down long before the stationary phase is reached and continues with a lengthened *m.g.t.*, as in Fig. 1 for the growth in valine.

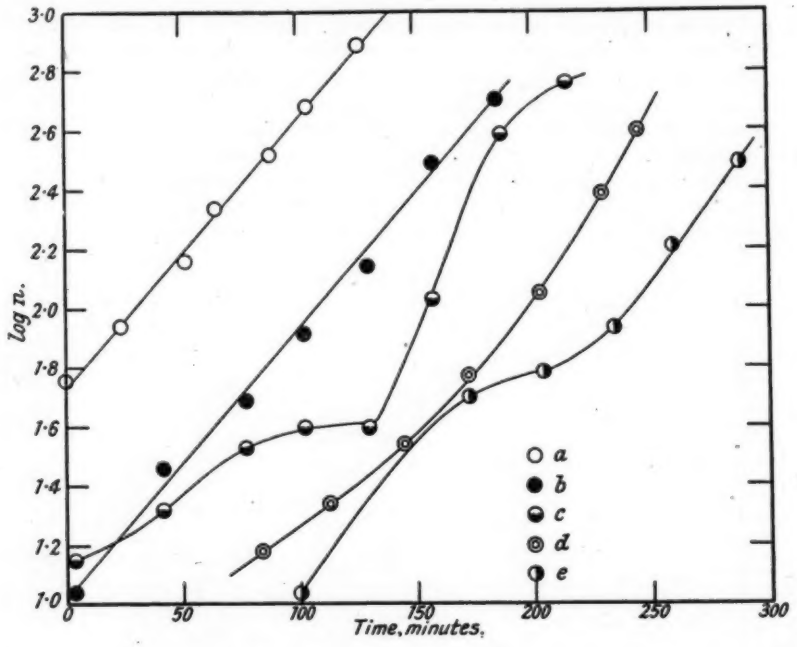
*Discussion.*—The facts described above are best interpreted in the light of the hypothesis that growth depends upon a delicately balanced series of reactions, one or other of which may become rate-determining according to circumstances.

In bouillon the *m.g.t.* is only 18′. In glucose–phosphate media with ammonium sulphate or amino-acids, this rate is not nearly reached, slower processes being rate-determining. In growth, something must be derived from the carbon source and something from the nitrogen source, and the intermediate derived from one of the sources will tend to be more readily available than that from the other. Since the growth rates with different nitrogen sources form a fairly compact group, one may conclude that the nitrogen-containing intermediate is the more readily available. In so far as the mutation of the organism tended somewhat to increase the differences between the *m.g.t.*'s with various nitrogen sources, it would mean that the relative rates of the two classes of reaction have been slightly shifted.

With some unaerated amino-acids growth ceases early: this effect is best explained by assuming the formation of an inhibitor, removable by aeration. The hypothesis that this removal is due to an oxidative mechanism developed *within the cell*, helps to link together a number of facts. It ascribes the change in reponse to aeration (after the minor mutation of the organism) to an improvement in an intracellular mechanism. Logically this

change could equally well be explained by assuming that a different and more easily removable inhibitor is produced after mutation.

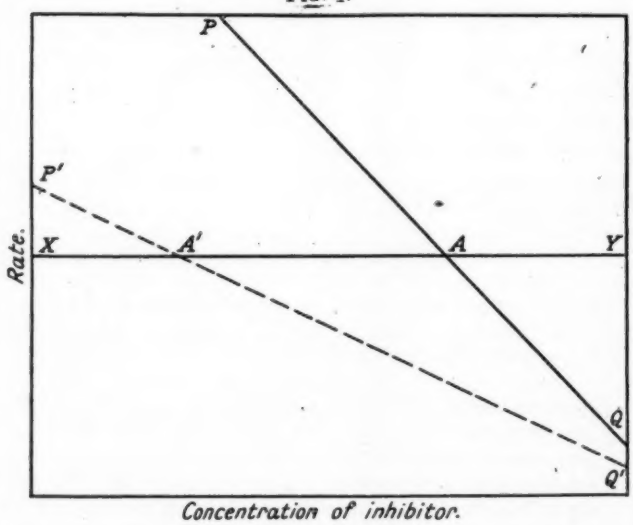
FIG. 3.




The first hypothesis, however, has the advantage of offering also a partial explanation of the anomalous growth curves referred to above (Fig. 3).

Suppose among the growth-determining processes there are two, with rates depending upon the concentration of a certain inhibitor as in Fig. 4. If the pair are affected by the inhibitor according to the curves $XY$ and $PQ$, the former will be rate-determining until the concentration of inhibitor corresponds to the point $A$, after which the other takes control. If $PQ$ is steep, and especially if, by the time $A$ is reached, the number of organisms is large, the rate then falls off very rapidly and there is an almost abrupt transition to the stationary phase. This is a common occurrence. If, however, the two reactions correspond to $XY$ and $P'Q'$ then the deceleration is more gradual, since it sets in at $A'$, which is reached when there are fewer cells and the rate of inhibitor formation is still small. Such a state of affairs may well correspond to the valine type of curve (Fig. 1).

FIG. 4.




The cell mechanisms responsible for the removal of this inhibitor may themselves well depend upon the building up of intermediate products. The removal reaction could therefore itself show a lag and we might have the abortive setting in of a stationary phase, subsequently disturbed by the development of the removal reaction. This state of affairs seems to correspond to curve (*c*) in Fig. 3. What determines the occasional lag in the inhibitor-removing mechanism is not clear: it may be immeasurably small traces of impurity such as grease. The fact that the temporarily inhibited growth sometimes makes up for lost time as in Fig. 3 (*c*) is interesting, and could be explained as follows. During normal growth a steady concentration of some intermediate substance is established: to this corresponds the normal *m.g.t.* The inhibitor now comes into play and prevents the utilisation, but not the formation, of this intermediate, which therefore builds up to an abnormally high concentration in the cell. As soon as the inhibitor-removing reaction comes into play the intermediate begins to be utilised again and, being at

an abnormally high concentration, gives rise to an abnormally high growth rate until the normal balance is restored.

The type of curve shown in Fig. 3 (*d*) will depend upon the gradual development of the inhibitor-removing reaction.

That the same organism may show the various types of curve in an apparently random manner may be explained by the influence of uncontrollably small amounts of impurities, but also by the fact that the state of the enzymes must be a function of the age and previous history of the cell and may therefore show variations from test to test great enough to influence the exact quantitative balance between these various delicately adjusted processes.

PHYSICAL CHEMISTRY LABORATORY, OXFORD UNIVERSITY. [*Received, January 5th*, 1943.]

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## 51. *Physicochemical Aspects of Bacterial Growth. Part IX. The Lag Phase of* Bact. Lactis Aerogenes.

By R. M. LODGE and C. N. HINSHELWOOD.

1. A method is worked out for determining from total counts of bacterial cultures the lag, *i.e.*, the time elapsing between inoculation and the establishment of the logarithmic growth phase.
2. When *Bact. lactis aerogenes* is inoculated in to an artificial medium containing asparagine as nitrogen source, the lag first increases regularly with the age of the inoculum, and then settles down to a more or less constant value.
3. When a medium containing ammonium sulphate as nitrogen source is used, the lag is considerable for very young cultures (" early lag "), falls rapidly to a minimum value approaching zero, and then increases again as in the asparagine medium (" late lag ").
4. The early lag in the ammonium sulphate medium is diminished by traces of asparagine, but only insofar as these are themselves enough to support some independent growth. It is diminished by lowered concentration of glucose in the medium.
5. Sterile filtrates from an actively growing culture approaching the minimum lag (para. 3) remove the early lag. This shows that a diffusible lag-removing substance is produced in the solution.
6. This being so, the lag should be less, the greater the number of cells transferred in the inoculum, even when the amount of the old medium transferred is kept constant (as can be arranged by the use of a centrifuge). This is confirmed, and an approximate quantitative expression for the early lag can be established on the assumption that all cells make their contribution to a common store of the lag-removing substance.
7. With asparagine and with alanine the early lag is absent, and, correspondingly, the lag is independent of the inoculum size.

THE lag phase of bacterial cells is known to depend upon their age (Penfold, *J. Hyg.*, 1914, **14**, 215; Stern and Frazier, *J. Bact.*, 1941, **42**, 479). The object of the experiments to be described was to study quantitatively the rate of development of lag with age.

We define lag as follows: $n_0$ is the initial number of living cells, $n$ the number after a total time $t$, and $k$ the growth rate constant. When logarithmic growth has once started, $n_0$ increases to $n$ in a time $t_1$ given by $2{\cdot}303 \log n/n_0 = kt_1$. The lag is taken as $(t - t_1)$. If the transition from lag phase to logarithmic phase is not abrupt, we still define the lag formally by this relation.

*Method.*—The culture used was *Bact. lactis aerogenes*, grown at 40·0° in various media, heart broth, the glucose–phosphate–ammonium sulphate medium described in Part I (J., 1938, 1930), and a similar medium in which ammonium sulphate was replaced by asparagine or alanine. The synthetic media will be referred to briefly as ammonium sulphate medium, alanine medium, and asparagine medium, respectively. Counts were made by hæmocytometer as previously described. " Aerated " media are those through which a gentle stream of air of normal carbon dioxide content was bubbled.

*True and Apparent Lag.*—Direct viable counts could not be made in the number and with the accuracy required. Total counts of living and non-living cells were therefore made. This raises the following problem. If the total initial number of cells is $n_0'$ of which $n_0$ are living, then from the definition given above, the time taken for $n_0$ to increase to $n_0'$ will be reckoned as part of the lag, giving an apparent value greater than the true one. Before proceeding, it was necessary to investigate the effect of ignoring the difference between $n_0$ and $n_0'$.

*Indirect method of determining viable counts and distinguishing between true and apparent lag.* Let $n_0$ be initial number of living cells, $x_0$ the number of dead cells, $n$ the total number at time $t$, and $L$ the true lag. Neglecting the death of organisms during the period of growth itself, which will usually be small, we have $n = x_0 + n_0 e^{k(t-L)}$, whence

$$\mathrm{d} \ln n/\mathrm{d}t = (1/n)\mathrm{d}n/\mathrm{d}t = kn_0 e^{k(t-L)}/n = k(n - x_0)/n$$

If all the cells were living $\mathrm{d} \ln n/\mathrm{d}t$ would be $k$: in general we call it $k_{\text{apparent}}$ ($k_{\text{app.}}$) and find $k_{\text{app.}} = k(1 - x_0/n)$ or

$$x_0/n = (1 - k_{\text{app.}}/k) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

The true and apparent mean generation times are in the inverse ratio of the corresponding values of $k$.

Usually, growth curves are measured over the range where $n$ is very much greater than $n_0$ or $x_0$, so that the apparent *m.g.t.* is indistinguishable from the true value, as shown by equation (1). If, however, we work with a

very large inoculum, and if an appreciable fraction of the cells are dead, then $k_{app.}$ will vary with $n$ and the growth curve will no longer be logarithmic. From the value of $k_{app.}$ at any value of $n$, the value of $x_0$ can be calculated. Two examples of the application of this method follow.

In an experiment with a bouillon culture which showed lag, the real *m.g.t.*, determined from measurements with small inocula, was 22·2 mins. When bouillon was given a heavy inoculum (hæmocytometer count = 48), the growth curve was logarithmic with an apparent *m.g.t.* of 22·7 mins. at $n = 90$. From equation (1) it follows that $x_0 = 2$, or that 96% of the inoculum was living.

In the second example a slightly different procedure was followed. The experiments were made in an aerated asparagine medium, in which the inoculant had developed a lag of 300 mins. Growth curves were determined for a series of inoculum sizes, and the apparent *m.g.t.* values obtained from the slope of the curves of log $n$ against $t$, at $n = 100$ and $n = 220$ respectively. The following table gives these values together with those calculated from equation (1) according to two hypotheses : (*a*) that the lag is real and the culture wholly living ($x_0 = 0$), and (*b*) that the true lag is zero and that the apparent lag is entirely due to the discrepancy between the total initial count and $n_0$. It is evident from the results that hypothesis (*a*) is very near the truth, *i.e.*, that a high percentage of the inoculum consisted of living cells.

| Inoculum size. | Apparent *m.g.t.* $n = 220$. | Calc. (*a*). $n = 100$. | Apparent *m.g.t.* $n = 220$. | Calc. (*b*). $n = 100$. | Observed *m.g.t.* $n = 220$. | $n = 100$. |
|---|---|---|---|---|---|---|
| 6·5 | 29·3 | 29·3 | 30 | 31 | 28 | 28 |
| 18 | 29·3 | 29·3 | 32 | 35·5 | 29 | 29 |
| 54 | 29·3 | 29·3 | 39 | 64 | 31·5 | 31·5 |
| 171 | 29·3 | 29·3 | 130 | — | 33 | 33 |

One may conclude, therefore, that with these cultures, typical of those which are not too old, the apparent lag really represents the delay in the onset of the logarithmic phase.

This ceases to apply when the cultures are very old or when they are kept in an unsuitable medium. The following experiments illustrate the fact that the very long lags sometimes observed with sub-cultures from such a medium are fictitious. An unaerated ammonium sulphate culture was inoculated at different ages into the aerated asparagine medium. Inocula of increasing age showed rapidly lengthening apparent lags, and finally appeared to have become quite sterile. The size of living inoculum from which growth could have occurred during the apparent lag period was calculated. It was found that the last culture to grow before sterility was reached would have grown from something of the order of a single cell. Thus one appeared to have been measuring, not the lengthening of the true lag, but the death rate of the organisms, since the hypothesis that this was occurring led to the prediction that the culture would become sterile at the observed point. Some typical results are given below : .

| Series I. | | | Series II. | | |
|---|---|---|---|---|---|
| Age, mins. | Appt. lag, mins. | Fraction assumed alive. | Age, mins. | Appt. lag, mins. | Fraction assumed alive. |
| 1220 | 20 | 0·63 | 720 | 17 | 0·68 |
| 1490 | 30 | 0·50 | 1023 | 60 | 0·23 |
| 1880 | 103 | 0·09 | 1515 | 750 | $3 \times 10^{-3}$ |
| 2274 | 860 | $2 \times 10^{-9}$ | 2130 | inf. | 0 |
| 2982 | inf. | 0 | | | |

In these experiments the inoculum size was 1·5 (hæmocytometer reading) which corresponds to $5 \times 10^7$ organisms per culture tube : thus the calculated living count of the last tube to grow was 0·1 cell in series I and 1·5 cells in series II. Since there are statistical fluctuations in death rate when the numbers are small, these results may be taken to represent fairly enough the passage over the border between viability and sterility.

With the above methods of discrimination available, it is believed that the lags recorded in the following sections truly represent the times taken for the living cells to develop from the resting phase to the phase of logarithmic growth.

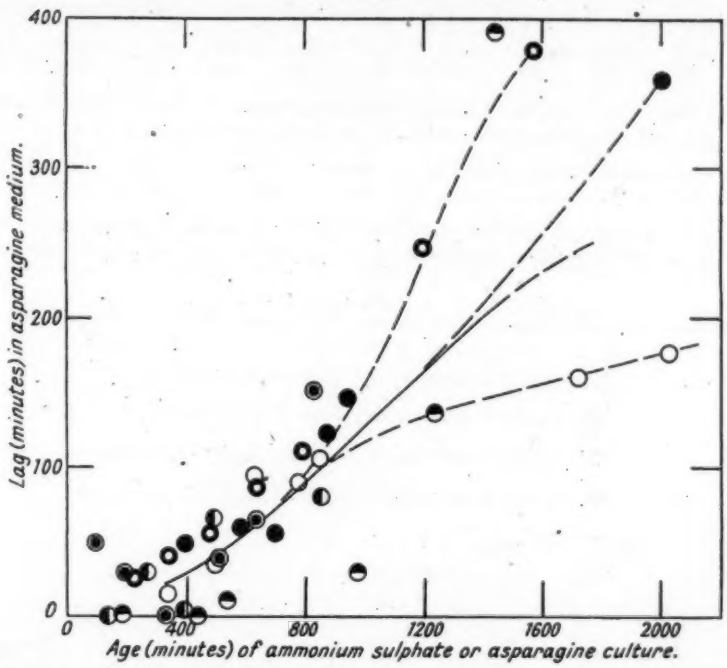
*Development of the Lag with Time.*—(a) *Early stages of development.* In the early stages the lag is fairly reproducible, and seems to be the same definite function of the age of the culture whether the ageing takes place in an asparagine or in an ammonium sulphate medium (Figs. 1 and 2). The ages are measured from the times at which the experimental count had the arbitrary value unity, which in fact corresponds fairly closely to the beginning of the logarithmic phase.

The lag measured in the ammonium sulphate medium at 40·0° is, for very young cells, of a considerable length, falls in the course of about 500 mins. to nearly zero, and then increases again rather rapidly. The phenomena occurring before and after the minimum will for brevity be referred to as early lag and late lag, respectively.

When cultures, whether aged in the asparagine or in the ammonium sulphate medium, are inoculated into asparagine there is little or no sign of early lag. The lag starts from zero for the youngest inocula and at an age of about 1500 mins. has increased to about 240 mins. In the ammonium sulphate medium in the 1500 mins. of ageing subsequent to the minimum the lag increases to about 500 mins., *i.e.*, it develops rather more rapidly than the lag measured in asparagine.

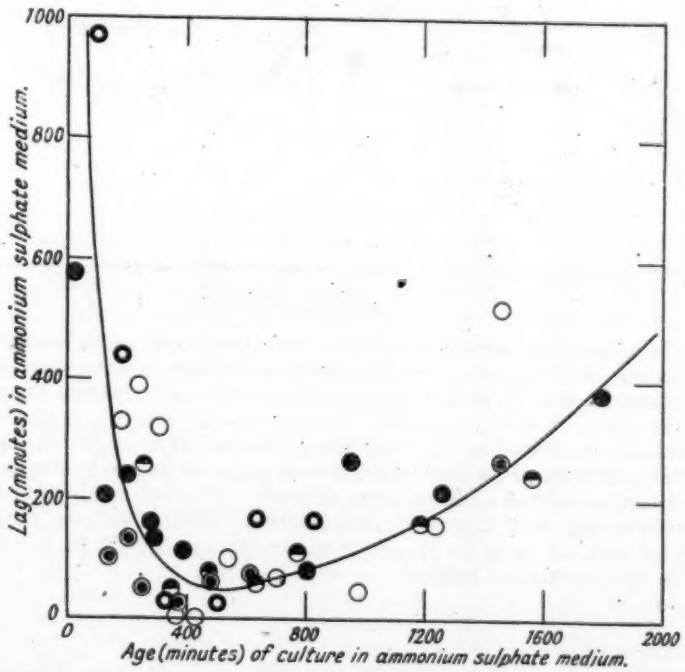
The development of the lag, as measured in the asparagine medium, is progressive during the period of growth of the parent culture and becomes slower as the stationary phase sets in. Qualitatively at least there

FIG. 1.




*Black-centred circles, Horizontally divided circles* } *cultures ageing in ammonium sulphate medium.* *Others: cultures ageing in asparagine medium.*

FIG. 2.




*Black-ringed circles, Open circles, Full circles* } *culture ageing in ammonium sulphate medium.* *Half-shaded circles, Black-centred circles* } *culture ageing in asparagine medium.*

is a correspondence between the development of the lag in the new medium and the cessation of active growth in the old. During the stationary phase the cells shrink in size: during the lag they swell again, as has been noted by other authors working with other organisms.

A few experiments were made with cells grown in and sub-cultured for test in an alanine medium. The general behaviour was similar to that found in the asparagine medium as the following numbers show :

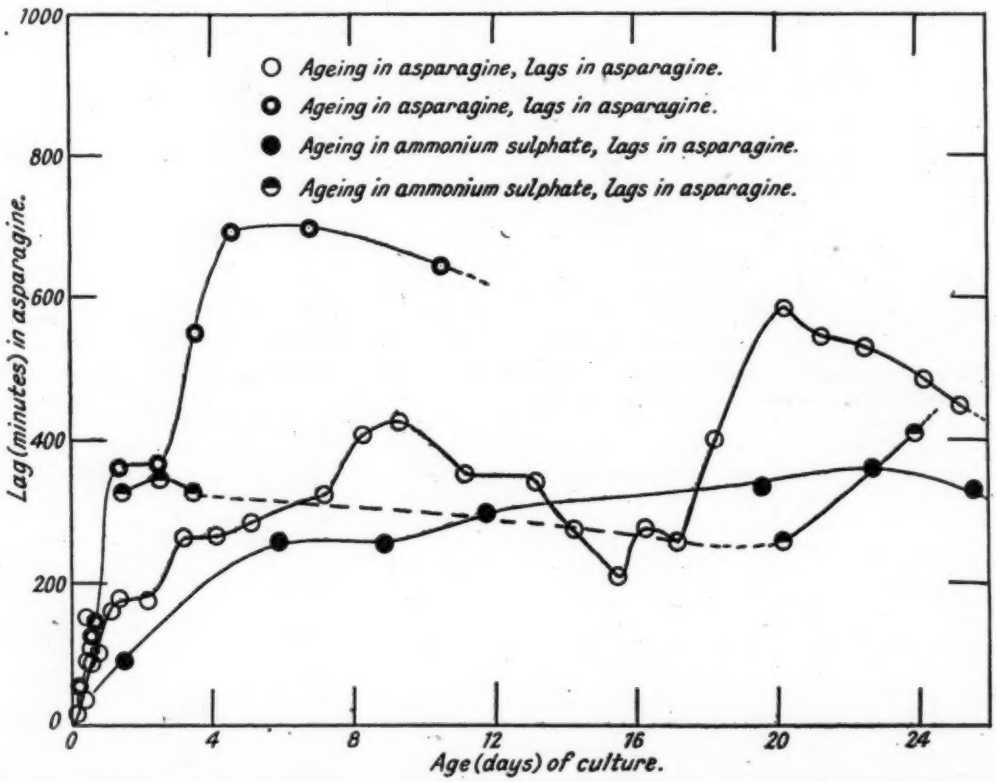
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age (mins.) ... | 220 | 282 | 387 | 480 | 615 | 720 | 915 | 1465 | 3125 | 4410 | 5730 | 7372 | 8095 |
| Lag (mins.) ... | 28 | 28 | 39 | 29 | 49 | 40 | 19 | 146 | 282 | 512 | 646 | 640 | 558 |

The rate of development of lag (measured in ammonium sulphate medium) when the bacteria aged in heart broth is indicated by the following numbers (means of several determinations) :

| | | | |
|---|---|---|---|
| Age (days) ..... | 1·0 | 2·0 | 4·0 |
| Lag (mins.) ..... | 110 | 210 | 380 |

(b) *Later stages of development.* These are shown in Fig. 3. The lag is less reproducible than in the early stages. It rises rapidly at first and then settles down to a more or less constant level about which it shows

FIG. 3.




irregular fluctuations, due to causes at present unknown. This level itself varies from culture to culture within certain limits, as may be seen in the figure. Although the operation of an unknown factor is evident, it is also clear that the cells tend to settle down to a constant resting condition—a state from which they must be brought before division will again occur. [Observations have been published (Martin, *J. Gen. Physiol.*, **1932, 15,** 691) which indicate that there are two phenomena at least in lag : in the initial stages the bacteria appear to be in a resting condition with a very low oxygen consumption rate per g. of bacterial nitrogen. In the later stages oxygen consumption rate increases, but division is still delayed. This question will be returned to later.]

*Factors influencing Early and Late Lag in the Ammonium Sulphate Medium.*—(1) *Glucose concentration.* Both early and late lag are reduced when the glucose in the medium is diluted, the former relatively more than the latter, as is shown by the numbers in Table I.

TABLE I.

| | | | | | | |
|---|---|---|---|---|---|---|
| Early lag : age of inoculant 200 mins. | | | | | | |
| Concn. of glucose (relative) ..... | 1·0 | 0·8 | 0·6 | 0·4 | 0·2 | 0·05 |
| Lag (mins.) ..... | 837 | 763 | 661 | 510 | 253 | 241 |
| Late lag : age of inoculant 28 hours. | | | | | | |
| Concn. of glucose (relative) ..... | 1·0 | 0·8 | 0·6 | 0·4 | 0·2 | 0·05 |
| Lag (mins.) ..... | 1281 | 1132 | 918 | 920 | 921 | 916 |

(2) *Filtrate from a young culture.* The early lag is reduced or even abolished by the addition of a filtrate, sterilised by heating to 100°, from young actively growing cells. An aerated ammonium sulphate culture was

divided into two parts, one being filtered and sterilised. The other was used as inoculant for more ammonium sulphate medium to which various amounts of the filtrate were added. The results are in Table II. In a second experiment (see Table III) an ammonium sulphate culture which had just grown to the point of zero lag was filtered, and the filtrate added to media with a very young inoculant.

TABLE II.

| Vol. of inoculum, 0·1 c.c. | | | | | |
|---|---|---|---|---|---|
| Vol. of filtrate, c.c. | 0 | 0·1 | 0·25 | 0·5 | 1·0 |
| Lag (mins.) | 182 | 106 | 46 | 70 | 12 |

TABLE III.

| Filtrate, c.c. | 0 | 0 | 0·1 | 0·5 | 0·75 | 1·0 |
|---|---|---|---|---|---|---|
| Lag (mins.) | 243 | 376 | 160 | 170 | 180 | 47 |

In contrast with this, the filtrate appears to have comparatively little influence on the late lag, the cause of which must be different (see Table IV).

TABLE IV.

| Filtrate from young ammonium sulphate cultures: inoculant 3 days old. | | | | | | | |
|---|---|---|---|---|---|---|---|
| Filtrate, c.c. | 0 | | | 0·1 | 0·5 | 1·0 | 1·5 |
| Lag (mins.) | 480 | 560 | 605 | 545 | 585 | 495 | 630 |
| **Filtrate from cultures of zero lag: inoculant 1 day old.** | | | | | | | |
| Filtrate, c.c. | 0 | | | 0·1 | 0·1 | 1·0 | |
| Lag (mins.) | 470 | 470 | 459 | 410 | 410 | 392 | |

(3) *Size of inoculum.** The above results indicate that a diffusible substance is produced during growth which can remove early lag. We should therefore expect the lag to depend upon the inoculum size: first, because some of the original medium is transferred with the inoculum, and, secondly, because larger numbers of organisms will more rapidly build up the required concentration of the lag-removing substance in the new medium. These expectations are confirmed.

Aerated ammonium sulphate culture was used as inoculant (*a*) at count $n_1 = 132$ and (*b*) at count $n_1 = 960$: the following results were obtained. (The "calc." values in Table V refer to the theory outlined in the next section.) $v$ is the volume in c.c. of inoculum and $n_0$ the initial count.

TABLE V.

| | (*a*). | | | (*b*). | | |
|---|---|---|---|---|---|---|
| $v$. | $n_0$. | Lag (obs.). | Lag (calc.). | $v$. | $n_0$. | Lag (obs.). | Lag (calc.). |
| 0·1 | 0·57 | 185 | 185 | 0·01 | 0·40 | >450 | 645 |
| 0·2 | 1·13 | 0 | 0 | 0·03 | 1·20 | 260 | 260 |
| 0·3 | 1·70 | −30 | 0 | 0·10 | 4·0 | 50 | 50 |
| 0·5 | 2·83 | − 8 | 0 | 0·20 | 8·1 | 10 | 0 |

In the above experiments the amount of medium transferred increased in proportion to the number of cells in the inoculum. To investigate the effect of number while the amount of active substance was kept constant, an aerated ammonium sulphate culture was grown to a small count and then centrifuged. The clear medium was removed by a pipette, and the residue shaken to give a heavy suspension. By mixing the latter with the clear medium in different proportions for use as inoculum, $n_0$ could be varied while $v$ was kept constant. The results are in Table VI.

TABLE VI.

| $n_0$ | 2·54 | 0·338 | (0·161 | 0·127) | 0·144 | (mean) | 0·055 |
|---|---|---|---|---|---|---|---|
| Lag (obs.) | 136 | 804 | (1030 | 970) | 1000 | (mean) | 1220 |
| Lag (calc.) | 136 | 640 | | | 950 | | 1220 |

Volume of inoculum 0·1 c.c. throughout.

Even with comparatively old ammonium sulphate cultures the lag is still dependent upon the inoculum size:

| Vol. of inoculum, c.c. | 0·005 | 0·01 | 0·02 | 0·03 | 0·04 | 0·11 | | 0·22 |
|---|---|---|---|---|---|---|---|---|
| Lag (mins.) | 3000 | 3000 | 1500 | 3000 | 1320 | 920 | 760 | 625 |

(4) *Additions of asparagine.* The early lag is reduced by small additions of asparagine to the ammonium sulphate medium: $w$ is the weight in g. $\times 10^{-6}$ of asparagine added to the 26 c.c. of culture solution.

| $w$ | 0 | | | | 2·5 | 5 | 15 | 50 | 100 | 250 | 500 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lag (mins.) | 400 | 404 | 427 | 444 | 435 | 387 | 377 | 260 | 120 | 90 | 90 |

* Compare other works on inoculum size from different points of view: Penfold, *loc. cit.*; Mueller, *Proc. Soc. exp. Biol. Med.*, 1939, **40**, 632.

The reduction seems, however, only to become marked when the asparagine is concentrated enough to contribute independently to the growth: there is no early lag in asparagine and the cells which it allows to grow themselves contribute to the removal of the lag in the others.

| | | | |
|---|---|---|---|
| $w$ ..... | 10 | 100 | 500 |
| $n_{24}$ ..... | 26 | 47 | 170 |

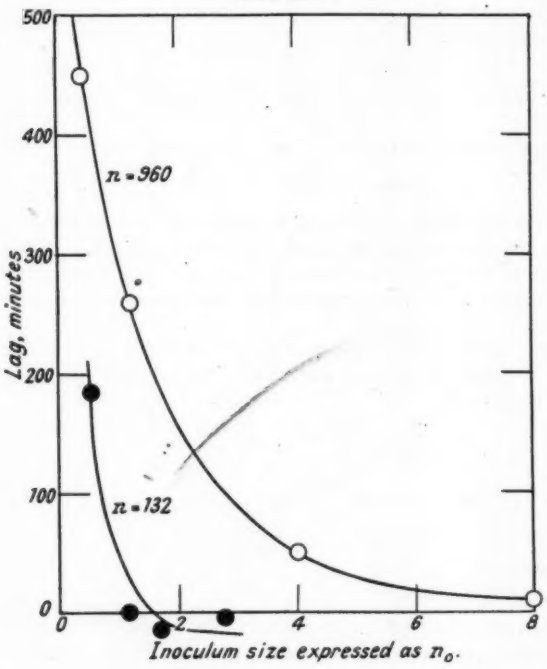
$n_{24}$ is the count after 24 hours' growth in a medium containing the asparagine with no other nitrogen source. It may be compared with $n_{24} = 2000$ for the standard ammonium sulphate medium.

*Theory of the Early Lag in Ammonium Sulphate Medium.*—We suppose that the lag ends when the concentration $c$ of some active substance reaches in each cell a critical value $c'$. We write:

$$c = \alpha v + \beta n_0 t + \gamma t$$

where $v$ is the volume of old medium transferred with the inoculum, and $\alpha v$ the concentration of the active substance thereby set up: $\alpha$ will be a function of the count $n_1$ of the inoculant medium (so long as the cells have not been separated from their original medium by centrifuging or otherwise), and $n_0$ is the number of cells per c.c. of the new medium, so that $\beta n_0 t$ is the contribution to $c$ which they have made in time $t$ by generating active substance in the medium. $\gamma t$ represents the concentration built up in a given cell without the contribution of the others. (The simple summation of the last two terms is an approximation, but will not be far from the truth.) When $c = c'$, $t = L$, whence

FIG. 4.




$$L = (c'/\beta - \alpha v/\beta)/(n_0 + \gamma/\beta) \quad . \quad . \quad (2)$$

From this equation we deduce the following: (*a*) When $n_0$ is constant but $v$ varies, as when filtrate is added, the lag should decrease linearly with increase of $v$; this is at least qualitatively true (see Tables II and III). (*b*) When $v$ is constant and $n_0$ varies (centrifuge experiments above), $L$ assumes a form such as

$$L = 382/(n_0 + 0{\cdot}258) \quad . \quad . \quad . \quad (3)$$

which has been used to express the results in Table VI. (*c*) In Table V, $n_0$ and $v$ vary together: moreover, $\alpha$ will be different for Table V (*a*) and Table V (*b*). Taking from (3) the value of $\gamma/\beta$, we can express the results in Table V (*b*) by $L = (448 - 2350v)/(n_0 + 0{\cdot}258)$. From Table V (*a*), to give the correct value for the lag for the smallest $n_0$, $\alpha/\beta$ must be taken as 2950. Taking $c'/\beta = 448$ from Table V (*b*) and combining it with (3), we find $\alpha/\beta = 660$. As the various calculated numbers show, these assumed constants reproduce the results in the tables as nearly as can be expected, so that we may regard the relative values of $\alpha/\beta$ as approximately correct. $\beta$ should be a constant but $\alpha$ should depend upon the amount of active substance in the inoculant medium. It should therefore vary with the count $n_1$ of the latter. The relation found is:

| | | | |
|---|---|---|---|
| $n_1$ ..... | 25 | 132 | 960 |
| $\alpha/\beta$ ..... | 660 | 2950 | 2350 |

This would indicate that the amount of active substance in the medium increases at first and then remains steady or declines, as would be expected on general grounds and from the fact that the lag increases again after a certain stage.

*Contrast between Ammonium Sulphate and Asparagine or Alanine.*—The early lag was little in evidence in the asparagine or alanine media. In confirmation of the views of the last section, the lag has been shown to be independent of the inoculum size with these media. An aerated asparagine medium was made, at two different ages, the inoculant of a new asparagine medium, with the following results:

| Count of parent medium. | $n_0$ for new medium. | Lag, mins. | Count of parent medium. | $n_0$ for new medium. | Lag, mins. |
|---|---|---|---|---|---|
| 1960 | 24·9 | 56 | 3500 | 29·6 | 350 |
| | 8·3 | 40 | | 14·8 | 490 |
| | 2·5 | 28 | | 5·9 | 350 |
| | 0·83 | 50 | | 1·8 | 338 |
| | | | | 0·6 | 345 |

Similar results were found with a young culture from alanine transferred to fresh alanine.

*Discussion.*—During the early lag a diffusible substance appears in the solution: it may be transferred with inocula or with sterile filtrates and reduce lag in fresh media. Each cell helps to end the lag of the others, whence the inverse relation, in ammonium sulphate media, of inoculum size and lag. Since early lag is not found in asparagine or alanine media, the active substance can plausibly, though not necessarily, be assumed to be a nitrogen compound, synthesised easily from amino-acids but less easily from ammonium sulphate.

The ending of the lag phase must depend upon two separate factors: the cells must begin to increase in size, and the growing cells must become capable of dividing. Either of these processes might become the rate-determining one. Which is the more important in the above experiments is not yet clear: the matter is under investigation.

After passing through a condition in which they show no lag, the cells age and the lag increases. The ageing may involve the decay of an active substance, but morphological changes occur also. The cells shrink in size, and there may be more profound internal changes. The curve showing the development of late lag could be interpreted as the decay curve of a chemical substance in the cells, but since filtrate has little effect on late lag, the structural changes must also have a profound influence.

The development of the lag does not continue indefinitely, but there is a tendency for it to settle down and then fluctuate about a mean which is maintained over a fairly long period. The existence of something like a limiting state is more striking than the fact that it is not a particularly stable one. Some hypothesis is needed to explain why there is this roughly steady value and why it changes with very slight changes of conditions. One may suppose that during the development of the lag various chemical and structural changes occur in parallel, and that the chemical balance of the organism in the steady, resting state depends upon the relative speeds of these processes. This same balance determines the leeway to be made up when the cell is placed in a new medium and hence the actual magnitude of the lag. This also is a starting point for further investigation.

PHYSICAL CHEMISTRY LABORATORY, OXFORD UNIVERSITY. [*Received, January 5th*, 1943.]

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## 52. *Physicochemical Aspects of Bacterial Growth. Part X. Observations on the Variability of Growth Rate of* Bact. Lactis Aerogenes.

By R. M. LODGE and C. N. HINSHELWOOD.

On storage for some months in a meat extract medium, a strain of *Bact. lactis aerogenes* showed in synthetic media (*a*) an increased growth rate, (*b*) an increased stationary population, and (*c*) a greater response to aeration.

The changed growth rate could not be traced to any variations in experimental technique.

Eleven sub-strains derived from single cells showed no significant differences. This shows that the strain was not sufficiently inhomogeneous to make selection a likely explanation of the change. The bacteria had apparently undergone true variation, involving a delicate readjustment of balanced intracellular reactions.

Attempts to induce variation in artificial media were negative, the growth rate in a standard medium being uninfluenced by 30—50 subcultures in various other media either favourable or unfavourable for growth.

THE growth of a particular strain of *Bact. lactis aerogenes* in a synthetic medium containing, apart from the unavoidable minimal traces of impurities, only glucose, potassium dihydrogen phosphate, ammonium sulphate, sodium hydroxide, and magnesium sulphate was under observation over a period of four years. During this time, as indicated in Part VIII, certain slight, but definite differences in behaviour appeared. The time taken for the number of organisms in the above medium (aerated) to double—the "mean generation time," *m.g.t.*—decreased by about 25%: the stationary population in the aerated medium increased to about double.

That variations in growth rate do in fact occur with this organism was shown by obtaining three different strains of *Bact. lactis aerogenes* from the National Collection of Type Cultures. Of these, one (NCTC. 99) grew on the standard aerated glucose–ammonium sulphate medium with *m.g.t.* = 41 mins., another (NCTC. 240) with *m.g.t.* = 35 mins., and the third (NCTC. Morris) was only cultured with difficulty.

Changes in growth rate may arise by the operation of any or all of three causes, which will now be discussed in turn.

(1) *Selection.*—A change from slower to faster growth rates, though not the reverse, can be expected if a culture is liable at any time to produce cells which divide at an abnormal rate, since the faster-growing types will multiply more quickly and displace the slower. From the usual straight-line nature of the curves obtained by plotting the logarithms of the numbers of bacteria per c.c. (log $n$) against time, we may deduce that if a given bacterium has a *m.g.t.* below the average, it will tend to produce daughter cells having *m.g.t.*'s above the average, and *vice versa*. Barber (1908) and others have shown that the individual division times of bacteria are not identical. Generally, however, the variability in this respect must average out. On the other hand, as shown in Part VIII, Fig. 3, curves of log $n$ plotted against time are not always linear, so that one cannot rely on this argument against selection by variation.

To investigate how easily selection might occur with *Bact. lactis aerogenes*, a culture grown in a medium containing a small amount of potassium cyanide which might enhance any possible inhomogeneity of the population, was plated out on nutrient agar. Discrete colonies from the agar grew after about 24 hours, and some of these, each presumed to have originated from a single bacterium, were transplanted into "heart broth," a meat extract of unknown composition. The mean generation times in the standard aerated medium of the

cultures so obtained were determined in duplicate. The results are given in Table I, where it will be seen that the average variation in the *m.g.t.*'s of all the cultures does not differ significantly from the average variation between pairs of cultures derived from the same colony. In fact, in the experiment described, no selection could be detected.

TABLE I.

| Colony number. | *M.g.t.*'s in aerated $(NH_4)_2SO_4$ (mins.). (1). | (2). | Diff. of (1) and (2) halved. | Diff. from the average, 31·9. (1). | (2). |
|---|---|---|---|---|---|
| 1 | 31·2 | 32·0 | 0·4 | 0·7 | 0·1 |
| 2 | 31·2 | 33·0 | 0·9 | 0·7 | 1·1 |
| 3 | 36·0 | 32·6 | 1·7 | 4·1 | 0·7 |
| 4 | 32·8 | 31·0 | 0·9 | 0·9 | 0·9 |
| 5 | 31·6 | — | — | 0·3 | — |
| 15 | 30·6 | — | — | 1·3 | — |
| 16 | 30·6 | 35·4 | 2·4 | 1·3 | 3·5 |
| 17 | 29·8 | 27·4 | 1·2 | 2·1 | 4·5 |
| 18 | 36·6 | 31·8 | 2·4 | 4·7 | 0·1 |
| 19 | 28·0 | 31·6 | 1·8 | 3·9 | 0·3 |
| 21 | 31·0 | 31·7 | 0·4 | 0·9 | 0·2 |
| 25 | 34·1 | 32·0 | 1·0 | 2·2 | 0·1 |

Average *m.g.t.* for all determinations = 31·9.
Mean variation of all cultures = 1·57.
Mean variation of pairs of cultures = 1·31.

(2) *Variation.*—It is well known that bacteria are able to change their characteristics of growth when stored on certain types of media. The bacteria in question had been stored on heart broth, subcultures being performed monthly. The regular transfer of the bacteria through a medium so favourable for growth might have been responsible for the change in growth rate, on account perhaps of an alteration in the relative predominance of some sets of intracellular reactions. It was thought of interest to attempt to influence this artificially. A systematic investigation of the growth rates, in a standard medium, of bacteria subcultured regularly in various favourable or unfavourable media was therefore undertaken.

*Bact. lactis aerogenes* was subcultured daily in several different media, and from time to time the *m.g.t.* was determined by inoculation from one of the serial cultures into the standard medium. The results, summarised in Table II, show that there is no obvious systematic change in the mean generation time with the serial number of the culture (col. 4), and that any trends lie within the average variation of growth rate determinations.

TABLE II.

*Summary of experiments on serial cultures.*

| 1. Medium in which serial culture was performed. | 2. Serial culture number. | 3. *M.g.t.*'s determined in the standard aerated medium (mins.). | 4. Average *m.g.t.* in standard aerated medium. As shown. | 5. Of all expts. in set. |
|---|---|---|---|---|
| *A.* Lactose, $KH_2PO_4$, $(NH_4)_2SO_4$, $MgSO_4$. (Unaerated.) 3 April—2 May | 25 | 24·0, 23·6, 25·3 | 24·3 | |
| | 30 | 23·2, 28·6, 29·0 | 26·9 | 25·6 |
| *B.* Glucose, $KH_2PO_4$, $(NH_4)_2SO_4$, $MgSO_4$. (Aerated.) (Standard medium.) 9 May—25 June | 2 | 26·3, 26·3, 26·3 | 28·3 | |
| | 5 | 30·2, 30·4, 30·4 | | |
| | 6 | 30·5 | 28·3 | |
| | 7 | 30·5 | | |
| | 11 | 24·5 | | |
| | 12 | 27·6 | | |
| | 16 | 27·8 | 29·0 | |
| | 18 | 28·3 | | |
| | 19 | 26·0 | | |
| | 42 | 34·0 | | 28·5 |
| *C.* Glucose, $KH_2PO_4$, $(NH_4)_2SO_4$, $MgSO_4$, KCN. (Unaerated.) 15 July—20 August | 6 | 35·0, 35·0 | 35·0 | |
| | 31 | 33·8, 33·8 | 33·8 | |
| | 32 | 33·0, 33·6 | 33·3 | 34·0 |
| *D.* Heart broth, plus ethyl alcohol (2%). (Unaerated.) 1 July—1 September | 15 | 33·3, 31·4 | 32·3 | |
| | 48 | 32·6, 35·4 | 34·0 | 33·2 |

On the other hand, the *average* mean generation times for the various inoculants (col. 5) show variations between themselves of significant magnitude. (It appears that, here, there was a slow change in the parent strain, kept in bouillon, from which the series A, B, C and D were derived at intervals over the period of about five months.)

(3) *Experimental Conditions.*—A carefully standardised technique was employed for the determination of bacterial growth rates. If the change apparently undergone by the bacteria is to be attributed to an alteration in experimental conditions, some quite subtle refinement must be involved. The only uncontrolled factors recognised in the experimental conditions were (*a*) the age of the inoculum, (*b*) the rate of aeration, and (*c*) the source of the air.

The effects of these factors on the growth rate were accordingly investigated. Table III shows that there is no significant change in growth rate with the age of the inoculum. Table IV shows that the rate of aeration

TABLE III.

*Effect of the age of the inoculant on the mean generation time in aerated medium.*

Inoculant: Unaerated heart broth.

| Age of inoculant, days. | hours. | *M.g.t.* (mins.). | Average *m.g.t.* | Age of inoculant, days. | hours. | *M.g.t.* (mins.). | Average *m.g.t.* |
|---|---|---|---|---|---|---|---|
| 0 | 5·5 | 31·6 | 30·9 | 9 | 13 | 29·4 | 31·1 |
| 0 | 9·5 | 30·2 | | 28 | 12 | 31·8, 32·2 | |
| | | | | 58 | 0 | 29·2, 33·8 | 31·5 |

Average *m.g.t.* = 31·2 mins.

TABLE IV.

*Effect of aeration rate and air source on the mean generation time.*

Mean generation times in mins.

| | Fast aeration: >10 bubbles per sec. | Slow aeration: 1 bubble per sec. |
|---|---|---|
| Filtered fresh air from outside the laboratory | 28·5 | 29·0 |
| Filtered air from the compressed air supply | 31·4 | 31·5 |

has no effect on the growth rate between the limits studied. All growth curves discussed above were obtained with aeration rates between these wide limits. There is, however, a small difference in growth rate evident between the two sources of air supply, which may, perhaps, be attributed to a small discrepancy in their carbon dioxide contents (Gladstone, Fildes, and Richardson, *Brit. J. exp. Path.*, 1935, **16**, 335; cf. Part II, J., 1938, 1936).

Another point which militates against the assumption of any drift in experimental conditions is that the lags, as determined in the standard medium, of inocula grown in the standard medium (*B*, Table II), all fall on the same curve when plotted against inoculant age, irrespective of the serial culture number of the inoculant (Table V).

TABLE V.

*Culture ageing in, and lags determined in, the standard aerated ammonium sulphate medium.*

| Age of inoculum from $n = 1$, in hours. | Serial culture number. | Lag, mins. | Age of inoculum from $n = 1$, in hours. | Serial culture number. | Lag, mins. | Age of inoculum from $n = 1$, in hours. | Serial culture number. | Lag, mins. |
|---|---|---|---|---|---|---|---|---|
| 0·5 | 19 | 580 | 6·5 | 16 | 117 | 30 | 42 | 1050 |
| 2·2 | 12 | 204 | 13·5 | 11 | 88 | 32·5 | 33 | 1195 |
| 3·5 | 7 | 242 | 16 | 5 | 266 | 36 | 54 | 1160 |
| 4·7 | 2 | 161 | 21 | 11 | 214 | 55 | 6 | 667 |
| 5·1 | 18 | 133 | 30 | 22 | 1380 | | | |

Finally, the greater growth rates recorded during the period April—June 1942 (Table II) cannot be due to traces of unknown constituents carried over from the heart broth, since these must have been completely eliminated during the considerable number of serial subculture operations in synthetic media.

It therefore seems that the bacteria underwent a true variation when stored in the broth. Such changes in bacteria are thought to be due to an alteration in the rather delicate balance between enzyme reactions proceeding in the cells. It appears that such changes have occurred twice during the time that this particular strain of *Bact. lactis aerogenes* has been under observation. Such changes might be expected to be capable of artificial control; several attempts have been made to exercise such control but, so far, the results have been negative.

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## 53. *The Nitrosation of Phenols. Part XIX. The Three Cresols and their Methyl Ethers. Some Semicarbazide Reactions.*

By HERBERT H. HODGSON and ERIC A. C. CROUCH.

Nitrous acid reacts normally with *o*- and *m*-cresol to give the 5- and the 6-nitroso-derivative respectively, but it nitrates *p*-cresol to form 3-nitro-*p*-cresol. Nitrosylsulphuric acid nitrosates or nitrates *o*- and *m*-tolyl methyl ethers according to conditions, but not *p*-tolyl methyl ether. 5-Nitroso-*o*-cresol and *5-nitroso-o-tolyl methyl ether* are converted by dilute nitric acid into 3 : 5-dinitro-*o*-cresol and the *m*-tolyl isomerides are both oxidised to 6-nitro-*m*-cresol, *i.e.*, both nitrosotolyl methyl ethers lose the *O*-methyl group. Some analogy with the behaviour of the dialkylanilines towards nitrous acid is indicated. The differences between the nitroso-*o*- and -*m*-tolyl methyl ethers in their behaviour towards semicarbazide are discussed.

IN this investigation three nitrosation procedures have been used: (*a*) The standard process with sodium nitrite in dilute sulphuric acid (Bridge, *Annalen*, 1893, **277**, 85); (*b*) the aqueous acetic acid method; and (*c*) the nitrosylsulphuric acid method of Hodgson and Nicholson (J., 1939, 1808).

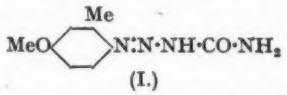
By (*a*) and (*b*), *o*- and *m*-cresol afforded 5-nitroso-*o*-cresol and 6-nitroso-*m*-cresol respectively, but *p*-cresol was nitrated to 3-nitro-*p*-cresol. Procedure (*c*) gave violent reactions with all three cresols and no useful products were obtained. All three tolyl methyl ethers were unaffected by (*a*) and (*b*), but (*c*) gave nitroso- or nitro-compounds with the *o*- and *m*-compounds according to the conditions, *viz.*, *5-nitroso-o-tolyl methyl ether* or 3 : 5-dinitro-*o*-cresol from the *o*-ether, and *6-nitroso-m-tolyl methyl ether* from the *m*-ether; *p*-tolyl methyl ether gave no useful product.

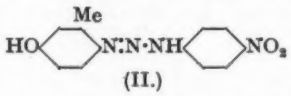
It will be noted that *p*-cresol is nitrated in the *o*-position to the hydroxyl group analogously to the *o*-nitration of dimethyl-*p*-toluidine (Hodgson and Kershaw, J., 1930, 277), whereas normal *p*-nitrosation occurs with *o*- and *m*-cresol. In the cases of the *o*- and the *m*-tolyl methyl ether, however, nitration, when it occurs, is accompanied by expulsion of the methyl from the methoxyl group as in analogous cases among the dialkylanilines (Hodgson and Nicholson, J., 1941, 470).

5-Nitroso-*o*- and 6-nitroso-*m*-cresol and 5-nitroso-*o*- and 6-nitroso-*m*-tolyl methyl ethers were readily oxidised by dilute nitric acid at 40° (cf. Robertson, J., 1902, **81**, 1477), the *o*-compounds giving 3 : 5-dinitro-*o*-cresol, and the *m*-compounds 6-nitro-*m*-cresol. It would appear, therefore, that, under the experimental conditions used, the action of nitrosylsulphuric acid on the cresols and their methyl ethers proceeds by nitrosation and subsequent oxidation, with expulsion of the *O*-methyl group from the ethers.

The position of the nitroso-group in the nitrosotolyl methyl ethers was determined by oxidation of the *m*-tolyl compound to the corresponding nitro-compound and by conversion of the *o*-tolyl compound into *p*-toluquinone.

According to Bamberger (*Helv. Chim. Acta*, 1931, **14**, 242), substituted hydrazines and aromatic nitroso-compounds react by initial addition of equimolecular amounts in a reaction of the aldol type, followed by oxidation of the resulting compound by another molecule of the nitroso-compound, which itself is reduced to a hydroxylamine. We find, however, that 6-nitroso-*m*-tolyl methyl ether condenses like a ketone with semicarbazide to form *4-methoxy-2-methylbenzenediazoaminocarbonamide* (I), which crystallises unchanged from


(I.)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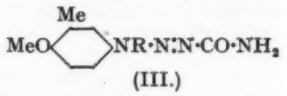
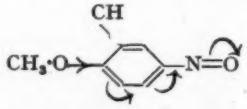

(III.)

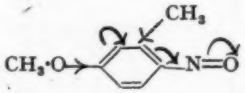
boiling aniline, and reacts rapidly with cold *p*-nitrophenylhydrazine to form *4′-nitro-4-hydroxy-2-methyldiazoaminobenzene* (II), the methoxyl group being demethylated. On the other hand, 5-nitroso-*o*-tolyl methyl ether reacts with semicarbazide according to the Bamberger generalisation; the product (III, R = OH) could not be crystallised and was identified by conversion by boiling aniline into *4-methoxy-3-methylhydrazobenzene-N-diazocarbonamide* (III, R = NHPh). Since only one oxygen atom (exclusive of the methoxyl oxygen) remains in the final compound, the reaction takes place by substitution at the N·OH group rather than by condensation at the CO·$NH_2$ group, which was found by Borsche and his co-workers (*Ber.*, 1901, **34**, 4299; 1904, **37**, 3177; 1905, **38**, 831; cf. also Wilson, Hopper, and Crawford, J., 1922, **121**, 866) to be the main reaction between semicarbazones and amino-compounds on heating:

$$CMe_2{:}N{\cdot}NH{\cdot}CO{\cdot}NH_2 \xrightarrow{RNH_2} CMe_2{:}N{\cdot}NH{\cdot}CO{\cdot}NHR + NH_3.$$

5-Nitroso-*o*-tolyl methyl ether condenses normally with *p*-nitrophenylhydrazine to form *4′-nitro-4-methoxy-3-methyldiazoaminobenzene*.

This difference in reactivity between the nitroso-*o*- and -*m*-tolyl methyl ethers may be ascribed to the difference in anionoid character of the *N*-oxygen atoms. In the *o*-tolyl compound the anionoid character is mainly due to methoxyl, the *C*-methyl being in the *m*-position to the nitroso-group, but in the *m*-tolyl ether





both groups exercise their full effect. Consequently, both hydrogen atoms of semicarbazide react with the *N*-oxygen in the *m*-tolyl ether, but only one in the case of the *o*-tolyl ether. Both hydrogen atoms of *p*-nitrophenylhydrazine react with the *N*-oxygen in each ether, but the reaction is much more intense (as it should be) with 6-nitroso-*m*-tolyl methyl ether, not only taking place in the cold but also expelling the methyl group from the methoxyl group. The same reactivity of the *N*-oxygen in 6-nitroso-*m*-tolyl methyl ether is seen in its behaviour with hydroxylamine, a diazo-compound being formed as shown by subsequent coupling with β-naphthol (Hodgson, J., 1931, 1494), whereas the *N*-oxygen in 5-nitroso-*o*-tolyl methyl ether fails to react.

## Experimental.

*Oxidation of 5-Nitroso-o-cresol and its Methyl Ether.*—The nitroso-compound (8 g.) was added in 30—40 minutes to well-stirred dilute nitric acid (60 c.c. of a solution of 50 c.c. of nitric acid, *d* 1·42, and 150 c.c. of water) at 40°. The precipitated 3 : 5-dinitro-*o*-cresol crystallised from dilute methyl alcohol (charcoal) in orange needles, m. p. 85—86° (Found: N, 14·1. Calc.: N, 14·1%).

*Action of Nitrosylsulphuric Acid on o-Tolyl Methyl Ether.*—(1) *Nitration.* A well-stirred solution of the ether (4 g.) in glacial acetic acid (25 c.c.) was maintained at 0° during the gradual addition of sodium nitrite (4 g.) in concentrated

sulphuric acid (10 c.c., *d* 1·84). The mixture was kept for 3 days at room temperature and then poured into water (600 c.c.). The precipitated 3 : 5-dinitro-*o*-cresol crystallised from dilute methyl alcohol (charcoal) in orange needles, m. p. and mixed m. p. 85·5—86°.

(2) *Nitrosation.* Sodium nitrite (8 g.) was slowly stirred into sulphuric acid (25 c.c., *d* 1·84) cooled in running water, and the mixture cautiously heated until the appearance of a yellow tinge (at 110°); it was then cooled rapidly to 0° with vigorous stirring. The thick paste was stirred gradually into a mixture of *o*-tolyl methyl ether (8 g.) and glacial acetic acid (25 c.c.) at − 10° to − 15°, the temperature not being allowed to rise above − 5°; the whole liquefied. (Nitrosation had occurred if a small quantity of the mixture gave a deep blue colour with cold water; a red colour indicated nitration, probably due to too rapid mixing.) After 10 minutes the mixture was run into ice-water. The precipitate of *5-nitroso-o-tolyl methyl ether* crystallised from light petroleum in pin-cushion-like clusters of green needles, m. p. 53·5° (Found : N, 9·3. $C_8H_9O_2N$ requires N, 9·26%), which were only slightly soluble in water but very soluble in methyl and ethyl alcohols and were oxidised slowly in the air to a brown powder.

6-Nitroso-*m*-cresol is oxidised by dilute nitric acid, as described above, to 6-nitro-*m*-cresol, m. p. 125° after crystallisation from benzene (Found : N, 9·3. Calc. : N, 9·15%).

*Action of Nitrosylsulphuric Acid on* m-*Tolyl Methyl Ether.*—(1) Nitration under the conditions described for *o*-tolyl methyl ether gave 6-nitro-*m*-cresol, m. p. and mixed m. p. 124°.

(2) Nitrosation as described above afforded *6-nitroso-*m-*tolyl methyl ether*, which crystallised from light petroleum in grass-green needles, m. p. 22° (Found : N, 9·4. $C_8H_9O_2N$ requires N, 9·3%), was very soluble in ethyl and methyl alcohols, but sparingly soluble in water, was oxidised by dilute nitric acid immediately to give 6-nitro-*m*-cresol, and gave an intense yellow-brown colour with concentrated sulphuric acid. The nitrosation mixture, when diluted with water, gave a red-violet colour.

*4-Methoxy-2-methylbenzeneazo-β-naphthol* was formed by heating a solution of 6-nitroso-*m*-tolyl methyl ether (2·5 g.), hydroxylamine hydrochloride (1·2 g.), sodium acetate (1·5 g.), and β-naphthol (2·4 g.) in ethyl alcohol (20 c.c.) and water (20 c.c.) for 30 minutes on the water-bath. It was precipitated with water at 0°, extracted with dilute sodium hydroxide solution, dissolved in hot ethyl alcohol, and the concentrated solution poured into water; the crystals that separated had m. p. 193° (Found : N, 9·3. $C_{18}H_{16}O_2N_2$ requires N, 9·6%).

*Action of Nitrous Acid on* p-*Cresol.*—A solution of *p*-cresol (4 g.), sodium nitrite (10 g.), and sodium hydroxide (2 g.) in water (100 c.c.) was treated gradually at 0° with dilute sulphuric acid (8 c.c., *d* 1·84, and 32 c.c. of water). After 30 minutes' stirring, the mixture was steam-distilled; 3-nitro-*p*-cresol crystallised from the distillate in yellow needles, m. p. 32·5° (Found : N, 9·3. Calc. : N, 9·15%). The same substance was obtained by the nitrosation method of Hodgson and Nicholson (J., 1939, 1808).

*Action of Semicarbazide on the Nitroso-*o- *and* -m-*tolyl Methyl Ethers.*—6-Nitroso-*m*-tolyl methyl ether (1·5 g.), semicarbazide hydrochloride (1·5 g.), sodium acetate (2 g.), and methyl alcohol (15 c.c.) were heated on the water-bath for 30 minutes. The resulting precipitate crystallised from pyridine in cream-yellow micro-plates of *4-methoxy-2-methylbenzenediazoaminocarbonamide*, m. p. 224°, and then from boiling aniline in pale yellow, lenticular plates, m. p. 230° (Found : N, 27·1. $C_9H_{13}O_2N_4$ requires N, 26·9%).

5-Nitroso-*o*-tolyl methyl ether, treated in the same way, yielded a brown precipitate, from a solution of which in boiling aniline (15 c.c.) *4-methoxy-3-methylhydrazobenzene-N-diazocarbonamide* separated in yellow needles, m. p. 238° (Found : N, 23·3. $C_{15}H_{17}O_2N_5$ requires N, 23·5%).

*Action of* p-*Nitrophenylhydrazine on the Nitroso-*o- *and* -m-*tolyl Methyl Ethers.*—5-Nitroso-*o*-tolyl methyl ether (2·5 g.), *p*-nitrophenylhydrazine hydrochloride (3·2 g.), and sodium acetate (2 g.) were heated with methyl alcohol (15 c.c.) and water (15 c.c.) on the water-bath for 15 minutes; the resulting *4′-nitro-4-methoxy-3-methyldiazoaminobenzene* crystallised in khaki-coloured needles, m. p. 187·5° (Found : N, 19·8. $C_{14}H_{14}O_3N_4$ requires N, 19·6%), which gave a wine-red colour with alcoholic potassium hydroxide.

When 6-nitroso-*m*-tolyl methyl ether was treated with the same reagents in the cold, a strong aromatic odour was perceptible; the reaction was completed by 5 minutes' warming in the water-bath. The resulting *4′-nitro-4-hydroxy-2-methyldiazoaminobenzene* crystallised from aniline in brick-red micro-plates, m. p 205° (decomp. beginning at 185°) (Found : N, 20·8. $C_{13}H_{12}O_3N_4$ requires N, 20·65%), which were slightly soluble in alcoholic potassium hydroxide, giving a golden colour.

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## 54. *The Cause of the Variation of the Colour of Crystals of Commercial Sodium and Potassium Ferrocyanide [Cyanoferrate(II)].*

By HERBERT E. WILLIAMS

The results of experiments on the action of light, heat, and air on solutions of the ferrocyanides [cyanoferrates(II)], acid, alkaline, and neutral, together with suggestions for the reactions taking place in the formation of pentacyanoaquoferrate-(II) and -(III) and ferricyanide [cyanoferrate(III)] are recorded. Methods for detecting and estimating small amounts of the aquo-salt and ferricyanide in ferrocyanide solutions are described. It is shown that the amber-coloured crystals sometimes found in commercial ferrocyanide are caused by the presence of traces of colloidal ferric hydroxide. Briggs's explanation that the amber colour is due to the presence of small quantities of pentacyanoaquoferrate(II), and Irving and Cherry's implied suggestion that it is caused by traces of colloidal Prussian-blue, are shown not to be correct for commercial potassium or sodium ferrocyanide.

THE colour of ferrocyanide crystals sometimes varies from light yellow to amber. To account for this variation, Briggs (J., 1911, **99**, 1019, 1038) suggested that there were two stereoisomeric forms differing in shade. It was shown later by Piutti (*Ber.*, 1912, **45**, 1831) that the absorption spectra of the supposed isomerides were indistinguishable, and by Bennett (J., 1917, **111**, 490) that the two types were of crystallographic identity and therefore could not be isomerides. Stereoisomerides, however, have been found amongst the organic ferrocyanides (Hartley, J., 1913, **103**, 1196) and are theoretically possible with the metallic salts. A close examin-

ation of commercial ferrocyanides manufactured in different ways, and of laboratory-prepared samples, has failed to detect any variations in the crystals except from the presence of small amounts of impurities. Briggs (J., 1920, **117**, 1026) has attributed the amber colour of the crystals to the presence of a small proportion of pentacyanoaquoferrate(II)—a strongly coloured substance—formed in acid solutions. These salts are isomorphous with the ferrocyanide of the same base and crystallise with it.

Amber-coloured crystals can be prepared as described by Briggs, by acidifying a solution of the ferrocyanide saturated at 40°, and allowing it to cool out of contact with air; they contain small quantities of the aquo-salt, varying from 0·5 to 0·8% of the potassium and 0·3 to 0·5% of the sodium ferrocyanide.

Pentacyanoaquoferrate(II) only occurs in ferrocyanide crystals obtained from acid solutions, or neutral solutions that have been exposed to light for some time. The prussiates of commerce are never crystallised from acid solutions, the liquor being kept alkaline to restrain decomposition. Pentacyanoaquoferrate-(II) or -(III) cannot exist in hot alkaline solution, being completely converted into ferro- or ferri-cyanide. It is obvious, therefore, that Briggs's explanation is not the complete solution of the problem.

Many amber-coloured crystals have been examined, including those obtained from solutions that had undergone long boiling, or been left exposed to light in alkaline solution. These sometimes contained a minute amount of aquo-salt, but the majority were entirely free from that compound. From a study of these crystals and the solutions from which they were obtained, it was concluded that the amber colour was due to traces of colloidal ferric hydroxide formed by the action of light or heat on the alkaline solution.

Irving and Cherry (J., 1941, 25) suggest that the amber colour found in some crystals of Bunsen's salt, $(NH_4)_4Fe(CN)_6,2NH_4Cl$, including those prepared by Briggs's method, may be due to the presence of a trace of colloidal Prussian-blue. Although this explanation may be possible in some cases for ammonium ferrocyanide and its double salts, owing to the somewhat different behaviour of solutions of this salt, it does not apply to the salts of alkalis for reasons given below; it also fails for amber crystals of the ammonium salt obtained from acid solutions, or neutral solutions that have been exposed to light, these crystals invariably containing the aquo-salt. As commercial ferrocyanide crystals have all been obtained from hot alkaline solutions, they must be free from Prussian-blue also; in fact, if a trace of Prussian-blue is added to a ferrocyanide solution so as to give a green tinge, a short exposure to light will precipitate red-brown ferric hydroxide: the same reaction will take place on heating. A freshly prepared solution of potassium or sodium ferrocyanide is faintly alkaline to phenolphthalein, and if such a solution containing a drop of the indicator solution is neutralised with dilute acetic acid until the colour is just discharged, and exposed to light and air, the solution will be coloured pink in 2—3 minutes' exposure, and deep red in an hour or so. If again neutralised and re-exposed, it again becomes alkaline. This series of operations may be repeated until ferrous ferrocyanide is precipitated. If the original neutralised solution is kept in the dark, no coloration develops after several days. The reaction is probably expressed as follows:

(1) $K_4Fe(CN)_6 + 2H_2O \rightleftharpoons H^{\cdot} + OH' + K^{\cdot} + K_3Fe(CN)_5 + H_2O + CN' \rightleftharpoons HCN + KOH + K_3[Fe(CN)_5,H_2O]$

(2) $12K_3[Fe(CN)_5,H_2O] + 4KOH + O = 10K_4Fe(CN)_6 + Fe_2O_3 + 14H_2O$

Baudisch (*Ber.*, 1929, **62**, 2706) found that pentacyanoaquoferrate(II) is produced when neutral solutions of ferrocyanide are exposed to light in the absence of air. In the presence of air the aquo-salt is oxidised to the violet aquo-salt, which reacts with ferrocyanide to give more complicated, pale yellow compounds. Baur (*Helv. Chim. Acta*, 1925, **8**, 403) obtained similar results, and concluded that the discoloration was due to the formation of colloidal ferric hydroxide.

Solutions of different ferrocyanides in different concentrations and acid, alkaline, or neutral, were exposed to the air and diffused daylight, and corresponding solutions exposed to the air in the dark. The solutions acidified with acetic acid and exposed to the light and air developed aquo-salt(II) and ferricyanide: after 6—7 weeks 70—75% of the ferrocyanide of a 10% solution was so converted. The amount of aquo-salt(II) increased with increasing amounts of acetic acid until sufficient was added to combine with one atom of the base of the ferrocyanide; further addition of acid had no accelerating effect, but increased the proportion of insoluble, green iron cyanogen compounds. In neutral solutions the same action takes place, much more slowly, and in alkaline solutions it is inhibited.

The rate of formation of the two salts in acid, neutral, or alkaline solution during 30 days' exposure to light and air is shown in the following results:

| | Aquo-salt (II), %. | Ferricyanide, %. |
|---|---|---|
| Acidified with HOAc ..... | 31·09 | 32·00 |
| With no addition ..... | 3·41 | 1·84 |
| Alkaline with $K_2CO_3$ ..... | 0·17 | 0·16 |

The above figures are calculated as the equivalent amount of ferrocyanide converted. The alkaline solution showed no change after 10 days, and only the slightest trace after 20 days.

In the acidified solution the rate of formation of these two salts was increased by exposing a larger surface of the solution to the air, or by blowing air through, which removed the hydrogen cyanide as it was formed, and oxidised the salt. In the dark, exposure to air caused the same reactions, but more slowly, those in the neutral solutions being negligible.

The formation of pentacyanoaquoferrate(II) in the acidified solution may be expressed as follows:

(3) $K_4Fe(CN)_6 + HOAc \rightleftharpoons KOAc + HK_3Fe(CN)_6 \rightleftharpoons K_3Fe(CN)_5 + H^+ + CN^- + H_2O \rightleftharpoons K_3[Fe(CN)_5,H_2O] + HCN$

When the warm, saturated, acidified solution is allowed to cool out of contact with air, as in Briggs's method, the aquo-salt(II) is found both in solution and in the crystals. The equivalent amount of free hydrogen cyanide may be estimated in the solution. No further change takes place on keeping during many weeks, and no ferricyanide is formed without exposure to the air. Ferro- and ferri-cyanides and pentacyanoaquoferrate-(II) and -(III), together with a small green precipitate of iron cyanogen compounds, were the only compounds found in these solutions.

When excess of potassium ferrocyanide solution is shaken with some violet aquo-salt, the latter is immediately reduced, and an equivalent quantity of ferrocyanide is oxidised to ferricyanide. In the absence of ferrocyanide, ferricyanide oxidises aquo-salt(II) to the violet aquo-salt(III). As judged from the colour of the solutions, it would appear that but little of the violet aquo-salt(III) existed in solution until the ferrocyanide content became small. When the solution is precipitated with lead acetate, the lead ferrocyanide carries down with it about 2—3% of its weight of the aquo-salt(II), any remaining aquo-salt(II) being converted into the violet-salt(III) by the ferricyanide if present. The reaction

(4) $K_3Fe(CN)_6 + K_3[Fe(CN)_5,H_2O] \rightleftharpoons K_4Fe(CN)_6 + K_2[Fe(CN)_5,H_2O]$

proceeds from left to right in the absence of ferrocyanide and in the reverse direction if it is present in excess. The pentacyanoaquoferrate(II) precipitated with the lead ferrocyanide may be completely recovered as the violet aquo-salt(III) by washing it with a solution of a ferricyanide containing lead acetate. If the ferricyanide in the original solution is in excess of the yellow aquo-salt(II), none of the latter will be found in the precipitated lead ferrocyanide.

An examination of the potassium and sodium ferrocyanide of current British and Continental makes has shown that they seldom contain more than a very slight trace of aquo-salt(II), the majority being entirely free from it. Traces of carbonylferrocyanide [pentacyanocarbonylferrate(II)] are very occasionally found in potassium ferrocyanide crystals obtained by extracting the cyanogen compounds from "spent oxide" or "cyanogen mud"—products obtained in the purification of coal gas—with lime.

Analysis of five different makes of commercial potassium ferrocyanide, both British and Continental, are given below:

| | 1. | 2. | 3. | 4. | 5. |
|---|---|---|---|---|---|
| Potassium carbonylferrocyanide, % | — | 0·04 | — | — | 0·23 |
| Potassium pentacyanoaquoferrate(II), % | — | — | 0·03 | — | — |
| Potassium chloride, % | 0·05 | 0·04 | 0·05 | 0·02 | 0·19 |
| Potassium sulphate, % | 0·01 | sl. tr. | v. sl. tr. | — | tr. |
| Potassium carbonate, % | 0·05 | 0·01 | 0·02 | 0·007 | 0·14 |
| Potassium formate, % | — | — | — | — | — |
| Potassium thiocyanate, % | 0·005 | — | — | v. sl. tr. | 0·023 |
| Potassium ferricyanide, % | — | — | v. sl. tr. | — | — |

If the ferrocyanide solution is boiled with a little potassium cyanide and filtered and crystallised, very pale crystals are obtained as described by Briggs (*loc. cit.*); they are entirely free from aquo-salt(II), ferricyanide, or ferric hydroxide. In a similar manner, sodium ferrocyanide may be obtained in faint yellow, almost white, crystals. This reaction suggests that the normal colour of the crystals is partly due to occluded ferric hydroxide, and the variations in shade to differences in proportion.

The amber-coloured crystals are probably formed in the following manner. During the evaporation of the solution some decomposition always takes place, with formation of ferric hydroxide as described below. This hydroxide is first formed in colloidal solution and finally separates as a flocculent precipitate. When the hot solution is run into a crystalliser, the precipitate settles to the bottom or may be filtered off before crystallisation takes place, and crystals of the salt of normal colour separate on cooling. If the solution is left for some time, particularly in the presence of light and air, the cold solution darkens slightly owing to colloidal ferric hydroxide, as concluded by Baur (*loc. cit.*). The crystals partly dissolve and recrystallise owing to minor fluctuations of temperature and other causes, and after some days the larger crystals will be of an amber colour, particularly on the outer edges owing to occluded ferric hydroxide. If the solution is alkaline, these crystals will be free from aquo-salt(II) or ferricyanide. Amber-coloured crystals may be prepared by adding a dilute emulsion of Prussian-blue to a warm saturated solution of a ferrocyanide so long as it is decomposed on shaking, and allowing the filtered solution to crystallise.

A solution of ferrocyanide saturated with sulphur dioxide, exposed to light and air for several days, will be converted to the extent of 40—50% into pentacyanosulphitoferrate(II). This action takes place in the absence of light, but much more slowly. Similarly, exposure to diffused daylight of a ferrocyanide solution containing a little sodium nitrite produces nitroprusside, tests with ammonium sulphide being positive after ½ hour and strong after 3 hours. In the dark, the mixed solution does not respond to the test even after 24 hours. If the solution also contains aquo-salt(II), the test is positive in a short time in the light or the dark.

A cold solution of ferrocyanide, when made alkaline and shaken with some precipitated mercuric oxide

and protected from the light, forms a little mercuric cyanide, but when the filtered solution is exposed to the light, ferric hydroxide is rapidly precipitated, the solution becoming cloudy in about 30 seconds.

Light also has a powerful effect on the neutral or alkaline solutions of the ferrocyanides. A solution of the potassium salt, made alkaline with potassium hydroxide and exposed to direct sunlight, first darkens and then soon becomes cloudy, precipitating ferric hydroxide. A neutral solution behaves in a similar manner but more slowly. No ferricyanide or aquo-salt(II) is formed in alkaline solution, or, if formed, it is immediately decomposed. Both salts are, however, formed in the neutral solution. No such action takes place in the absence of light.

A solution of 2% each of potassium ferro- and ferri-cyanide, made alkaline with potassium hydroxide, rapidly decomposed in sunlight, ferric hydroxide being precipitated with reduction of the ferricyanide. A portion of the same mixture kept in the dark was unaltered after several days.

Ferricyanide solutions are rapidly reduced in the cold when exposed to light (Vogel, *Ber.*, 1871, **4**, 96). When a dilute solution of potassium ferricyanide containing lead acetate or cerous sulphate is exposed to direct sunlight, a cloudiness due to the formation of the corresponding ferrocyanide is observed in 3—4 minutes; a heavy deposit separates later.

Hauser and Biesalski's suggestion (*Ber.*, 1912, **45**, 3516) that the supposed isomeric " β " ferricyanide of Locke and Edwards (*Amer. Chem. J.*, 1899, **21**, 193,413) was due to occluded Prussian-blue in the crystals is possibly correct in some cases. Freshly prepared solutions of potassium ferricyanide are not alkaline to phenolphthalein, and do not become so on exposure to light or on heating. Nevertheless, Briggs's explanation that these darker crystals contain the violet aquo-salt(III) is in the majority of cases correct. Pentacyano-aquoferrate(III) will occur in the solution of the ferricyanide, not only after treatment by Briggs's method, but also on exposure of the neutral or acidified solution to the light, and by over oxidation in the preparation of the salt from ferrocyanide. The violet aquo-salt(III) may sometimes be found in the commercial salt, and crystals of the darker shade almost invariably contain this aquo-salt and not Prussian-blue.

Pentacyanocarbonylferrate(II) decomposes rapidly in dilute solutions when exposed to light, with precipitation of ferric hydroxide and conversion into normal ferrocyanide. Solutions of carbonylferrocyanides mixed with a little lead acetate or cerous chloride—the carbonyl salts of which are soluble—when exposed to light rapidly decompose with precipitation of the insoluble ferrocyanide and ferric hydroxide.

The violet ferric aquo-salt also fades and turns blue in the light, contrary to Lecocq's statement (*Bull. Soc. chim. Belg.*, 1911, **25**, 72). These reactions do not take place in the absence of light. A solution of pentacyanocarbonylferrate(II) acidified with acetic acid and kept in the dark, or more rapidly in the light, decomposes with replacement of the carbonyl by water to form the aquo-salt(II), together with ferrocyanide and a precipitate of Prussian-blue.

Cobalticyanide appears to be unaffected by light in either alkaline or neutral solution. In acid solution, a deeper yellow colour occurs, which is possibly due to the formation of some pentacyanoaquocobaltate(III).

Decomposition occurs when solutions of the ferrocyanides are boiled under atmospheric pressure, with loss of cyanogen and precipitation of ferric hydroxide. The extent of decomposition increases with dilution. This decomposition is inhibited by potassium hydroxide, carbonate, and cyanide. The solution of pure ferrocyanide after boiling usually contains some formate and carbonate, together with a little ferricyanide and a suspension of ferric hydroxide.

Dry crystals of ferrocyanide, protected from the air by a glass cover, are unchanged by light. Some dry crystals of the salt (2—3 lb.), removed from the top surface of a crystalliser and exposed to diffused daylight in a well-fitting, glass-covered case for 36 years, were entirely free from aquo-salt and ferricyanide or any other decomposition product. Dry samples of sodium, calcium, and magnesium ferrocyanide and of the sparingly soluble double salts of potassium and ammonium with calcium and barium ferrocyanide were similarly unchanged after 30 years in a corked bottle. Lithium ferrocyanide, however, changed owing to the difficulty of keeping the deliquescent crystals dry.

## Experimental.

*Analytical Methods.—Estimation of small quantities of aquo-salts in ferrocyanides.* (1) To 10—20 ml. of the ferrocyanide solution, add a little dilute ferricyanide (if not already present), 0·25—0·5 g. of dicyanodiamide, and 5 ml. of a 5% solution of sodium hydroxide, and heat nearly to the boil. A red colour develops in the presence of pentacyano-aquoferrate(II). No colour is obtained if ammonia is substituted for the sodium hydroxide, and sodium carbonate is not so efficient. The colour may be matched against a standard ferrocyanide solution containing a known quantity of the aquo-salt. This test will detect 1 part in 30,000 parts.

(2) To 10 ml. of the solution to be tested, add 10 ml. of 20-vol. hydrogen peroxide, followed by 5 ml. of glacial acetic acid. In the presence of a trace of the aquo-salt, a brown colour develops in a few minutes owing to formation of the strongly coloured aquo-salt(III). With larger amounts of the aquo-salt the colour is purple. With very slight traces the mixture should be allowed to stand for 10—15 mins. screened from the light and air. The colour may be matched against a standard. The sensitivity is the same as in (1).

(3) To 10 ml. of the solution to be tested, add 10 ml. of a 5% solution of sodium carbonate and a few drops of a 10% solution of thiocarbamide, and let the mixture stand for a few minutes. If aquo-salt is present a reddish-violet colour develops, and is matched against a standard. The method detects 1 part in 20,000 parts. If ferricyanide is absent a few drops of a 1% solution should be added before the thiocarbamide. This method, however, is not recommended for estimation, as the colour fades rapidly. Sato (*Biochem. Z.*, 1909, **23**, 44) gave this reaction as a delicate test for thiocarbamide, and stated that the addition of thiocarbamide to a solution of potassium ferrocyanide acidified with acetic or hydrochloric acid gives a green colour changing to blue, and that if sodium carbonate be substituted for the acid a reddish-violet colour develops in a few minutes. No such colorations occur with pure potassium ferrocyanide

solutions or even if they contain ferricyanide. The colorations are due to the presence of a trace of pentacyanoaquoferrate(II), and are only obtained with a ferrocyanide solution that has been exposed to light for some time or left acidified. The reaction is also given by a solution of pentacyanocarbonylferrate(II) or moist crystals that have been exposed to light. This is probably due to the replacement of carbonyl by water. The reaction is not given by the pure salt.

For the estimation of larger amounts of the aquo-salt(II), take 50 ml. of the solution, make it alkaline with sodium hydroxide, add a slight excess of hydrogen peroxide to decompose all ferricyanides, and boil the solution for 5 mins. All aquo-salt is decomposed by this method and converted into ferrocyanide with a quantitative precipitation of ferric hydroxide [equation (2)]. The latter is filtered off, washed, and estimated by any known method. The amount of ferric oxide obtained is multiplied by 24·075 to obtain the proportion of aquo-salt(II) in the solution taken, or by 31·65 for the equivalent of potassium ferrocyanide. The method is only moderately accurate. Pure potassium ferrocyanide, free from ferricyanide or aquo-salt, gives no precipitate of ferric hydroxide when boiled with the above reagents for 10 mins. if screened from the light.

*Detection and estimation of small quantities of ferri- in ferro-cyanide solution.* To 10 ml. of the neutral solution, add a few drops of pure 2 : 4-diaminophenol dissolved in glacial acetic acid. In the presence of a trace of ferricyanide a bright red colour is instantly formed. This may be matched against suitably prepared standards. This test will detect 1 part in 40,000 parts of solution.

*Estimation of total oxidised salt.* (1) To 10 ml. of the solution, add an excess of lead acetate and then potassium ferrocyanide until the supernatant liquor is a bright clear yellow, filter it, remove the excess lead with dilute sulphuric acid, and refilter it. The ferricyanide in the filtrate is estimated by known means. When the aquo-salt and ferricyanide are less than 3% of the ferrocyanide the addition of more ferrocyanide is unnecessary.

(2) To a measured quantity of the solution containing about 0·5 g. diluted to 500 ml., add 10 ml. of a saturated solution of zinc sulphate and a few drops of potassium iodide solution, and titrate the free iodine with 0·1 or 0·01N-sodium thiosulphate solution, using starch paste as indicator. There is no known means of separating pentacyanoaquoferrate(III) from ferricyanide.

The standard solution of the aquo-salt is prepared by dissolving a known quantity of pentacyanoaquoferrate(III) in a 10% solution of potassium ferrocyanide, the solution being kept protected from the light. The aquo-salt may be prepared by Cambi's method (*Gazzetta*, 1911, **41**, i, 157). The salt is then analysed, and a weighed portion dissolved in the 10% potassium ferrocyanide solution. For use as a standard, a measured portion is diluted with 10% solution of pure potassium ferrocyanide to the required volume.

*Other Details.*—The potassium ferrocyanide used in these experiments was a good commercial quality recrystallised from water twice, screened from the light, redissolved in warm water, and precipitated when cold by alcohol, dried, and kept in an amber bottle.

*Effect of surface area.* 100 Ml. of a 10% solution of the trihydrate were kept in (i) a cylinder and (ii) a dish of respective surface areas 15·02 and 60·1 sq. cm. for 7 days, and the content of ferricyanide was then (i) 0·220%, (ii) 0·952% : ratio of surface, 1 : 4; ratio of $K_3Fe(CN)_6$ formed, 1 : 4·21.

*Effect of concentration of ferrocyanide solution on the formation of ferricyanide when exposed to air.* The following data were obtained :

| Concn., %. | $K_3Fe(CN)_6$, % : after 7 days. | after 14 days. | after 21 days. |
|---|---|---|---|
| 1 | 2·24 (0·0224) | 5·6 (0·056) | 10·0 (0·1) |
| 5 | 0·614 (0·0307) | 1·344 (0·0672) | 2·0 (0·1008) |
| 10 | 0·448 (0·0448) | 0·728 (0·0728) | 1·17 (0·117) |

The figures in parentheses give the actual amount formed calculated as $K_4Fe(CN)_6.3H_2O$. 150 Ml. of 20% trihydrate solution kept in the dark for 14 days gave 0·03% of ferricyanide.

*Effect of acid concentration on formation of the aquo-salt in the light.* 100 Ml. of 10% ferrocyanide trihydrate gave the following percentages of aquo-salt with the stated amounts of acetic acid : 0·1 ml., 5·99; 0·5 ml., 17·65; 1·0 ml., 29·24; 2·0 ml., 42·67; 5·0 ml., 37·3; 10·0 ml., 37·2%.

100 Ml. of the same solution together with 1 ml. of acetic acid were exposed to light and gave the following data

| Time, days | 9 | 17 | 24 | 38 |
|---|---|---|---|---|
| Aquo-salt, % | 3·35 | 9·48 | 15·48 | 29·3 |
| $K_3Fe(CN)_6$, % | 9·52 | 14·84 | 22·8 | 37·3 |

*Decomposition during boiling at atmospheric pressure.* Various solutions were boiled, and the loss during successive periods of 10 hrs. determined :

| Solution. Loss, %, during : | 1st 10 hrs. | 2nd 10 hrs. | 3rd 10 hrs. |
|---|---|---|---|
| 1000 Ml. 10% $K_4Fe(CN)_6.3H_2O$ | 0·639 | 0·897 | 0·886 |
| 100 G. $K_4Fe(CN)_6.3H_2O$ in 200 ml. $H_2O$ | 0·095 | 0·12 | 0·101 |
| 25 G. $K_4Fe(CN)_6.3H_2O$ in 1200 ml. $H_2O$ | 2·319 | 2·17 | 2·318 |
| 1000 Ml. 10% $Na_4Fe(CN)_6.10H_2O$ | 0·482 | — | — |
| 100 G. $Na_4Fe(CN)_6.10H_2O$ in 200 ml. $H_2O$ | 0·052 | — | — |

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## 55. *The Stability of Racemates. Mandelic Acid and Some of its Derivatives.*

By W. Rogie Angus and R. P. Owen.

M. p. or f. p. curves for mixtures of the active and racemic forms of mandelic, acetyl- and propionyl-mandelic acids, and of methyl, ethyl, and *iso*butyl mandelates have been determined. Racemate stability is increased by acylation and by esterification.

In order to obtain some information regarding the stability of racemates of mandelic acid derivatives, the melting or freezing point curves of six compounds have been investigated.

### Experimental.

*Preparation of Materials.*—*r*-Mandelic acid was recrystallised from acetone–benzene. Its resolution was effected by *l*-ephedrine, Roger's modification (J., 1935, 1544) of Skita, Keil, and Meiner's method (*Ber.*, 1933, **66**, 979) being used.

In this way both *d*- and *l*-mandelic acid were obtained. The *l*-acid had m. p. 133·5°, $[\alpha]_{5461}^{20°}$ − 189·5° (*c*, 14·3 in acetone) {Skita *et al.* record m. p. 132·5—133·5°; $[\alpha]_{D}^{19°}$ − 155·3°; Roger (*loc. cit.*) gives $[\alpha]_{5461}$ in acetone − 189·8°, and (in J., 1932, 2168) $[\alpha]_{5461}^{20°}$ − 189·9° for pure *l*-acid.}

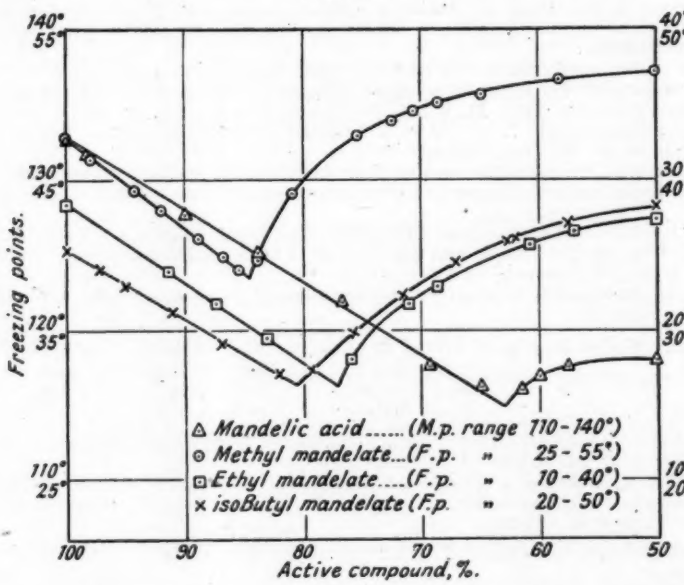
(a) O-*Acetyl*-r-*mandelic acid*. This was prepared by the method of Anschütz and Böcker (*Annalen*, 1909, **368**, 57). The needle crystals, obtained from water, are a dihydrate, m. p. 52°. Exhaustive desiccation gave the anhydrous form, m. p. 79°. [Anschütz and Böcker give m. p. 80°; Kaufler and Hertzog (*Ber.*, 1909, **42**, 3872) 76°; Savarian (*Compt. rend.*, 1908, **146**, 297) 79°; Dupont (*ibid.*, 1910, **150**, 1525) 74—75°].

(b) O-*Acetyl*-l-*mandelic acid*. Similarly prepared, this acid recrystallised from water as a monohydrate and on exhaustive desiccation gave the anhydrous acid, m. p. 96·8°, $[\alpha]_{5461}^{20°}$ − 194·1°, $[\alpha]_{D}^{20°}$ − 157° (*c*, 2·4 in acetone). {McKenzie and Humphries (J., 1909, **95**, 1106) also obtained a monohydrate and gave the m. p. of the anhydrous acid as 96·5—98°, $[\alpha]_{D}^{20°}$ − 157·7° (*c*, 2·2 in alcohol), $[\alpha]_{D}^{20°}$ − 153·7° (*c*, 2·058 in acetone); Walden (*Z. physikal. Chem.*, 1895, **17**, 706) gives $[\alpha]_{D}^{20°}$ − 156·4°.}

(c) O-*Propionyl*-r-*mandelic acid*. r-Mandelic acid (20 g.) and propionyl chloride (14 g.) were heated on a water-bath for 3 hours, excess propionyl chloride distilled off, and the resulting oil taken up in a little chloroform and precipitated as a solid by ligroin. On recrystallisation from water, the acid formed a dihydrate (m. p. indefinite, but about 50°); on recrystallisation from ligroin (b. p. 40—60°), 18 g. of anhydrous *propionyl*-r-*mandelic acid*, m. p. 51·2°, were obtained (Found: C, 63·35; H, 5·8. $C_{11}H_{12}O_4$ requires C, 63·5; H, 5·75%).

(d) O-*Propionyl*-l-*mandelic acid*. This *acid*, similarly prepared, had m. p. 70—71°, $[\alpha]_{5461}^{15°}$ − 172·4°, $[\alpha]_{D}^{15°}$ − 139·1° (*c*, 0·855 in carbon disulphide); $[\alpha]_{5461}^{15°}$ − 134·2°, $[\alpha]_{D}^{15°}$ − 124·5° (*c*, 0·466 in alcohol); $[\alpha]_{5461}^{15°}$ − 133·2°, $[\alpha]_{D}^{15°}$ − 107·5° (*c*, 0·327 in acetone) (Found: C, 63·3; H, 5·8%).

FIG. 1.




(e) O-*Benzoyl*-r-*mandelic acid*. r-Mandelic acid (20 g.) and benzoyl chloride (20 g.) were heated under reflux for 3 hours and subsequently treated as in (c). On recrystallisation from ligroin, 10 g. of anhydrous *benzoyl*-r-*mandelic acid*, m. p. 114—115°, were obtained (Found: C, 70·1; H, 4·7. $C_{15}H_{12}O_4$ requires C, 70·3; H, 4·7%).

*Esters*. These were prepared by the general method of Fischer and Speier (*Ber.*, 1895, **28**, 3254) and gave the following values for physical constants: Methyl r-mandelate, m. p. 58° [Zincke and Breuer (*Ber.*, 1880, **13**, 636) give 47—49°; Rupe (*Ber.*, 1895, **28**, 259) 52°; Acree (*Ber.*, 1904, **37**, 2767) 58°; Findlay and Turner (J., 1905, **87**, 752) 57°]. Methyl *d*-mandelate, m. p. 54—55° [Walden (*loc. cit.*) gives 55°; McKenzie and Wren (J., 1908, **93**, 312) 54·3°; Freudenberg and Markert (*Ber.*, 1925, **58**, 1753) 58°]; $[\alpha]_{5461}^{20°}$ + 272·6°, $[\alpha]_{D}^{20°}$ + 219·6° (*c*, 1·6 in carbon disulphide) (McKenzie and Wren give $[\alpha]_{D}^{20°}$ + 214° in carbon disulphide). Ethyl r-mandelate, m. p. 29° [Michael and Jeanpetre (*Ber.*, 1892, **25**, 1682) give 34°; McKenzie (J., 1899, **75**, 755) 37°; Findlay and Turner (*loc. cit.*) 29°]. Ethyl *l*-mandelate, m. p. 31° [Walden (*loc. cit.*) gives 35°; Walker (*Chem. Zentr.*, 1909, ii, 2113) 30°; Freudenberg and Markert (*loc. cit.*) 34°]. *iso*Butyl r-mandelate, m. p. 39° [Walden (*loc. cit.*) gives 35°]. *iso*Butyl *l*-mandelate, m. p. 35·8° [Wood, Chrisman, and Nicholas (J., 1928, 2186) give 35·5°]; $[\alpha]_{5461}^{20°}$ − 185·6°, $[\alpha]_{D}^{20°}$ − 149·7° (*c*, 1·27 in carbon disulphide) (Walden gives $[\alpha]_{D}$ − 144° in carbon disulphide; Wood *et al.* give − 124·58° for the molten ester).

*Freezing-point Curves*.—Although it was impossible to determine transition points, the f.-p. curves give an indication of the effect of substitution on the stability of the racemic forms; those for various mixtures of active and inactive compounds were obtained by the cooling curve method, and for all mixtures (except those of mandelic acid) the f. p.'s were taken instead of the m. p.'s. Only half of the full temperature–composition curve was determined, since the curves are symmetrical around the 50% co-ordinate.

(a) *Mandelic acid*. The f.-p. curve of this acid could not be determined because, on heating, it undergoes some form of internal condensation (see Note, this vol., p. 249). Instead, the m.-p. curve was determined by making artificial mixtures of the active and the racemic form and determining their m. p.'s by heating *once* only. The results are given in Table I and Figs. 1 and 2; the data from previous determinations [Adriani (*Z. physikal. Chem.*, 1900, **33**, 468) and Centnerszwer (*ibid.*, 1899, **29**, 715)] are included. The present results differ from those of the earlier workers in respect of the racemic portion. Each value now recorded has been confirmed several times and no reason can be given for the divergence. The eutectic point occurs at 114·8° and 63% of *l*-acid; Adriani gives 113° and 60% of *l*-acid.

*Acetylmandelic acid* (Table II and Fig. 1). The eutectic point is at 65° and the eutectic mixture contains 68·2% of *l*-acid.

*Propionylmandelic acid* (Table III; Fig. 1). Eutectic point, 37·8°; *l*-acid in eutectic mixture, 69·5%.

TABLE I.

| *l*-Acid, %. | Melting points. Adriani. | Centnerszwer. | Authors. | *l*-Acid, %. | Melting points. Adriani. | Centnerszwer. | Authors. |
|---|---|---|---|---|---|---|---|
| 100·0 | 133·0° | 132·7° | 133·0° | 70·0 | 118·2° | — | — |
| 98·2 | — | — | 131·8 | 69·2 | — | — | 117·5° |
| 95·0 | — | 129·9 | — | 65·0 | 115·8 | — | 116·1 |
| 90·0 | 128·1 | — | 127·7 | 62·5 | — | 117·9° | — |
| 87·5 | — | 126·7 | — | 61·7 | — | — | 116·0 |
| 83·8 | — | — | 125·1 | 60·0 | 113·0 | — | 117·0 |
| 80·0 | 123·2 | — | — | 57·6 | — | — | 117·5 |
| 76·9 | — | — | 121·8 | 55·0 | — | 119·2 | — |
| 75·0 | 120·6 | 120·6 | — | 50·0 | 118·0 | 121·0 | 118·0 |

FIG. 2.

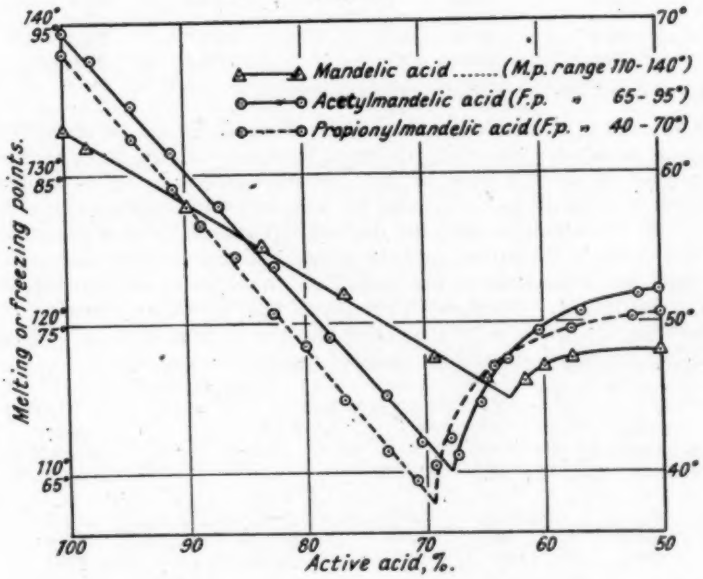



TABLE II.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *l*-Acid, % ..... | 100·0 | 97·86 | 94·70 | 91·30 | 87·40 | 82·40 | 78·25 | 73·60 |
| F. p. ..... | 94·6° | 92·6° | 89·6° | 86·4° | 83·8° | 78·8° | 74·0° | 70·0° |
| *l*-Acid, % ..... | 70·60 | 67·70 | 65·40 | 63·14 | 60·50 | 56·85 | 52·0 | 50·0 |
| F. p. ..... | 67·0° | 66·0° | 69·6° | 72·4° | 74·3° | 75·6° | 76·8° | 77·0° |

TABLE III.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *l*-Acid, % ..... | 100·00 | 98·21 | 94·56 | 92·10 | 88·80 | 86·00 | 82·83 | 80·10 | 77·00 |
| F. p. ..... | 68·0° | 65·0° | 62·4° | 60·0° | 56·5° | 54·4° | 50·6° | 48·4° | 44·6° |
| *l*-Acid, % ..... | 73·50 | 71·20 | 69·30 | 68·05 | 64·30 | 57·70 | 52·50 | 50·0 | |
| F. p. ..... | 41·4° | 39·4° | 40·4° | 42·2° | 47·0° | 49·4° | 50·2° | 50·5° | |

*Methyl mandelate* (Table IV; Fig. 2). Eutectic point 38·4°; eutectic composition, 84·5% of *l*-acid. The m. p. curve had been determined by Centnerszwer (*loc. cit.*) by taking the m. p.'s of artificial mixtures in capillary tubes, and his results are given for comparison. There is a great lack of agreement: Centnerszwer's values do not lie on a smooth curve; further, his m. p. for the racemic ester is 8° lower than ours, and the composition of his eutectic mixture considerably different. Although his m. p. curve should not coincide with our f.-p. curve there should be similarity in shape. Confirmation of each of our points several times, and comparison with the values for other esters, suggest that our curve is the more trustworthy.

TABLE IV.

| *d*-Ester, %. | F. p. Centnerszwer. | F. p. Authors. | *d*-Ester, %. | F. p. Centnerszwer. | F. p. Authors. | *d*-Ester, %. | F. p. Centnerszwer. | F. p. Authors. |
|---|---|---|---|---|---|---|---|---|
| 100·0 | 54·6° | 47·8° | 86·69 | — | 39·8° | 70·46 | — | 49·6° |
| 98·42 | — | 46·8 | 85·32 | — | 39·0 | 68·60 | — | 50·2 |
| 95·1 | 52·3 | — | 83·84 | — | 41·6 | 64·80 | — | 50·7 |
| 94·42 | — | 44·4 | 80·82 | — | 44·0 | 62·4 | 48·3° | — |
| 92·15 | — | 43·0 | 75·5 | 47·5° | — | 58·10 | — | 51·7 |
| 88·87 | — | 41·2 | 75·25 | — | 48·0 | 54·8 | 49·2 | — |
| 87·5 | 50·0 | — | 72·50 | — | 49·0 | 50·0 | 50·0 | 52·2 |

*Ethyl mandelate* (Table V; Fig. 2). Eutectic point 16·2°; eutectic composition, 76% of *l*-ester.

TABLE V.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *l*-Ester, % ..... | 100·00 | 91·40 | 87·40 | 83·18 | 76·10 | 71·03 | 68·48 | 60·80 | 57·10 | 50·00 |
| F. p. ..... | 28·4° | 24·0° | 21·8° | 19·4° | 18·0° | 21·8° | 23·0° | 25·8° | 26·6° | 27·4° |

iso*Butyl mandelate* (Table VI; Fig. 2). Eutectic point, 26·2°; eutectic composition, 80·5% of *l*-acid. Centnerszwer's m. p. data (*loc. cit.*) are given for comparison: they do not agree with ours.

TABLE VI.

| *l*-Ester, % | F. p. Centnerszwer. | F. p. Authors. | *l*-Ester, % | F. p. Centnerszwer. | F. p. Authors. | *l*-Ester, % | F. p. Centnerszwer. | F. p. Authors. |
|---|---|---|---|---|---|---|---|---|
| 100·0 | 35·3° | 35·2° | 82·1 | — | 27·0° | 62·7 | — | 35·8° |
| 97·6 | — | 34·1 | 79·4 | — | 27·3 | 62·6 | 37·8° | — |
| 95·2 | — | 32·8 | 75·6 | — | 29·8 | 62·0 | — | 36·0 |
| 95·0 | 33·3 | — | 75·0 | 37·0° | — | 57·4 | — | 37·1 |
| 91·3 | — | 31·2 | 71·4 | — | 32·2 | 54·7 | 38·2 | — |
| 87·3 | 32·9 | 29·0 | 67·0 | — | 34·6 | 50·0 | 38·7 | 38·2 |

All thermometers were checked against N.P.L. certified instruments.

*Discussion.*—The f.-p. curves of mandelic acid and its derivatives can be divided into two classes, *viz.*, those of acids and of esters, which are essentially different in shape. In the three acid curves the f. p.'s of the active acids are considerably higher than those of their racemic isomerides. The straight-line portion of these curves occupies the greater portion of the curve, and the area under the racemic curve is comparatively small. With the esters, the f. p.'s of the racemates are a few degrees higher than those of the active forms, the racemate portions occupy the greater part of the curves, and the areas under the racemic curves are comparatively large.

The three factors which are considered in examining the influence of substituents on the stability of the racemate are collected in Table VII. They are (i) the percentage of active isomeride in the eutectic mixture, (ii) the difference between the f. p. or m. p. of the active and the racemic form, $\theta_a - \theta_r$, and (iii) the difference between the f. p. or m. p. of the racemate and the eutectic temperature, $\theta_r - \theta_e$.

TABLE VII.

| | Active isomeride in eutectic, %. | $\theta_a - \theta_r$. | $\theta_r - \theta_e$. | | Active isomeride in eutectic, %. | $\theta_a - \theta_r$. | $\theta_r - \theta_e$. |
|---|---|---|---|---|---|---|---|
| *Acids*: | | | | *Esters*: | | | |
| Mandelic ..... | 63 | 15° | 3·2° | Methyl ..... | 84·5 | −4·4° | 11·8° |
| Acetylmandelic ..... | 68·2 | 17·6 | 12·0 | Ethyl ..... | 76·0 | −1·0 | 11·2 |
| Propionylmandelic ... | 69·5 | 17·5 | 12·7 | *iso*Butyl ..... | 80·5 | −3·0 | 12·0 |

There is a slight difference, about 2·5°, in $\theta_a - \theta_r$, on acylation of mandelic acid. The values for $\theta_r - \theta_e$, however, increase from 3·2° for mandelic acid to 12·0° for the acetyl and 12·7° for the propionyl derivative. The comparatively small value of $\theta_r - \theta_e$ and the fairly large value for $\theta_a - \theta_r$ in mandelic acid indicate that the transition temperature of the racemic compound into the *dl*-mixture is not far removed from the f. p. of the racemic acid. With the substituted acids, however, although the values of $\theta_a - \theta_r$ are practically the same as for mandelic acid, there is a considerable increase in the values of $\theta_r - \theta_e$ and also in the areas beneath the racemic curves, thus indicating that the transition temperatures of these racemates are not very near their f. p.'s.

The composition of the eutectic mixtures becomes progressively richer in the optically active component in passing from mandelic through acetyl- to propionyl-mandelic acid; this indicates that the stability of the racemic forms has been progressively increased. This fact is also borne out by the progressive increase in the areas occupied by the racemic portions. Further, the value of $\theta_r - \theta_e$ is greater for the propionyl than for the acetyl derivative. The view that this difference has a significant relation to the stability of the racemate and increases with increasing stability finds substantiation in the results for the esters.

From the data in Table VII it is clear that esterification greatly reduces the values of $\theta_a - \theta_r$ and that, for the three esters examined, the f. p.'s of the racemates are slightly higher than those of the active forms. Examination of Table VII and Fig. 2 shows immediately that esterification leads to an increased racemate area and to a eutectic mixture considerably richer in active component. Further, the comparatively large difference between $\theta_r$ and $\theta_e$ for the esters would appear to indicate that the transition points are far removed from the f. p.'s of the racemates. From all these considerations it is concluded that esterification increases racemate stability.

Possibly because one of the esters has a branched chain, the progressive variation, on ascending the homologous series, in the stability of the racemate and in factors related to the stability is not encountered. In passing from the methyl to the ethyl ester, however, there is a diminution in stability.

The results of Findlay and Campbell (J., 1928, 1768) on derivatives of tartaric acid are in general agreement with those now reported for mandelic acid.

The investigation reported in this paper was commenced many years ago by one of us (W. R. A.) at the suggestion of Professor A. Findlay, to whom our thanks are due for allowing us to continue the work and for much helpful advice in its early stages.

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR. [*Received, January 5th*, 1943.]

# 56. *Aqueous Solubilities of* r- *and* l-*Mandelic Acids and Three O-Acyl-*r-*mandelic Acids.*

By W. Rogie Angus and R. P. Owen.

The aqueous solubilities of *r*-mandelic, and of acetyl-, propionyl-, and benzoyl-*r*-mandelic acids at 5° intervals from 0° to 50°, and of *l*-mandelic acid from 25° to 70°, have been determined. Substitution causes a lowering of solubility, and that of the racemic acid is greater than that of the active acid at the same temperature. In geometrical and stereoisomerides the form with the lower m. p. has the greater solubility.

Existing data on the solubility of *r*-mandelic acid comprise isolated determinations at one temperature only. When these results are expressed in the same units they agree fairly well with each other and with those now communicated. Solubility, *S*, is expressed in g. per 100 g. of water.

| Temp. | Reference. | *S*. | Temp. | Reference. | *S*. |
|---|---|---|---|---|---|
| 20° | Lewkowitsch (*Ber.*, 1883, **16**, 1566) | 15·97 | 25° | Knox and Richards (J., 1919, **115**, 516) | 22·10 |
| | Schlossberg (*Ber.*, 1900, **33**, 1056) | 15·95 | | Ross and Morrison (J., 1933, 1016) ..... | 20·4 |
| | Authors ..... | 16·21 | | Authors ..... | 21·95 |
| 24·7 | Rimbach (*Ber.*, 1899, **32**, 2388) ... | 20·85 | | | |

Recorded values for the solubility of *l*-mandelic acid are somewhat higher than those now reported. They are: at 20°, Lewkowitsch (*loc. cit.*), 8·64; at 25°, Campbell and Garrow (*Trans. Faraday Soc.*, 1930, **26**, 560), 10·98; Ross, Morrison, and Johnson (J., 1937, 608), 12·68. No data are on record for the solubilities of the substituted acids.

## Experimental.

The acids were purified by the methods described in the preceding paper.

The solubilities of the racemic compounds were determined by a method closely resembling that of Dalman (*J. Amer. Chem. Soc.*, 1937, **59**, 2547). Round-bottomed glass tubes of 50-c.c. capacity, fitted with ground glass stoppers, were charged with 40 c.c. of water and sufficient excess of acid to ensure saturation at the appropriate temperature. The tubes were clamped to the rotating stirrer of an electrically-heated thermostat and rotated until equilibrium had been attained (10—20 hrs.). Samples were transferred at intervals to tared vessels, and analysed by titration with standard alkali of suitable concentration. Table I shows the solubilities of (*a*) *r*-mandelic acid, (*b*) acetyl-*r*-, (*c*) propionyl-*r*-, and (*d*) benzoyl-*r*-mandelic acids at various temperatures, $\theta$. Several values were obtained for each solute at each temperature; for *r*-mandelic acid the results were reproducible to 0·5%, and for the substituted acids to 1%.

Table I.

| $\theta$. | (*a*). | (*b*). | (*c*). | (*d*). | $\theta$. | (*a*). | (*b*). | (*c*). | (*d*). |
|---|---|---|---|---|---|---|---|---|---|
| 0° | 8·79 | 0·57 | 0·29 | 0·51 | 37° | 109·4 | — | — | — |
| 10 | 11·68 | 0·68 | 0·35 | 0·60 | 40 | 163·2 | 3·18 | 1·22 | 1·27 |
| 15 | 13·40 | 0·76 | 0·37 | 0·65 | 42·5 | 192·4 | — | — | — |
| 20 | 16·19 | 0·96 | 0·38 | 0·73 | 45 | 247·0 | 5·74 | 1·85 | 1·57 |
| 25 | 21·95 | 1·14 | 0·52 | 0·79 | 47 | 274·2 | — | — | — |
| 30 | 37·46 | 1·39 | 0·59 | 0·92 | 50 | 373·0 | 13·50 | 3·46 | 1·88 |
| 35 | 81·8 | 1·99 | 0·74 | 1·04 | | | | | |

The amount of *l*-mandelic acid available was relatively small and the solubility was, therefore, determined by Kurilоff's sealed-tube method (*Z. physikal. Chem.*, 1897, **23**, 547). The mean values of several observations at each temperature are given in Table II. Although the precision of this method is much less than that of the analytical method employed for the other acids, the results were reproducible to 2%.

Table II.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $\theta$ ..... | 24·5° | 27·5° | 31·5° | 37·0° | 41·5° | 44·0° | 46·5° |
| *S* ..... | 8·9 | 10·9 | 14·3 | 20·8 | 30·0 | 39·8 | 55·7 |
| $\theta$ ..... | 48·5° | 50·5° | 52·5° | 54·5° | 57·0° | 60·5° | 68·0° |
| *S* ..... | 74·0 | 94·4 | 112·8 | 129·1 | 155·1 | 194·0 | 289·2 |

All thermometers were checked against N.P.L. certified instruments.

*Discussion.*—The solubility curves of all these substances, except benzoyl-*r*-mandelic acid, show a similarity in shape. Up to a certain temperature (approx. 30—40°) the solubility increases slowly, but thereafter it increases rapidly with temperature. The substituted acids are much less soluble than mandelic acid, owing to acylation of the strongly hydrophilic hydroxyl group; and, in passing from acetyl- to propionyl-*r*-mandelic acid there is further diminution.

Both *r*- and *l*-mandelic acid have essentially similar solubility curves, but the solubility of the former is considerably greater than that of the latter at the same temperature and increases more rapidly with temperature. Scarcity of *l*-acid has prevented us from determining the solubility of the active forms of the other compounds.

The results for mandelic acid, however, fall into line with previous data on the solubility of geometrical and stereoisomerides in that the form with the lower m. p. is the more soluble; *e.g.*, for maleic (m. p. 130°) and fumaric (m. p. 300°) acids, Weiss and Downs (*J. Amer. Chem. Soc.*, 1923, **45**, 1003) give the solubility at 25°

as 78·8 and 0·70, respectively. Further, examination of Findlay and Campbell's data (J., 1928, 1768) on tartaric acid and tartrates reveals that, in each case, the more soluble form has the lower m. p. It is hoped to extend this investigation.

The authors thank Professor J. L. Simonsen, F.R.S., for his interest and encouragement.

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR. [*Received, January 5th,* 1943.]

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## 57. *The Interconversion of Hexœstrol and* iso*Hexœstrol.*

By D. A. PEAK and W. F. SHORT.

The dimethyl ethers of hexœstrol and *iso*hexœstrol are interconverted when heated at 305—310° in presence of hydrogen sulphide.

THE important œstrogen hexœstrol (Campbell, Dodds, and Lawson, *Nature*, 1938, **142**, 1121; Dodds, Golberg, Lawson, and Robinson, *Proc. Roy. Soc.*, 1939, *B*, **127**, 153; Campbell, Dodds, and Lawson, *ibid.*, 1940, *B*, **128**, 253) is conveniently prepared from its dimethyl ether obtained from *p*-α-bromopropylanisole by the action of sodium (Boots Pure Drug Co. Ltd., and W. F. Short, E.P. 523,320, 30.12.1938; Bernstein and Wallis, *J. Amer. Chem. Soc.*, 1940, **62**, 2871) or magnesium (Boots Pure Drug Co. Ltd. and W. F. Short, *loc. cit.*; Docken and Spielman, *J. Amer. Chem. Soc.*, 1940, **62**, 2163). Hexœstrol dimethyl ether prepared in this way is accompanied by *iso*hexœstrol dimethyl ether, which is demethylated to the relatively feeble œstrogen, *iso*hexœstrol. It was suggested by Dodds, Golberg, Lawson, and Robinson (*loc. cit.*) that hexœstrol and *iso*hexœstrol are the *meso*- and the racemic form respectively of γδ-di-*p*-hydroxyphenylhexane and this has been confirmed by the resolution of *iso*hexœstrol (Wessely and Welleba, *Naturwiss.*, 1940, **28**, 780).

*iso*Hexœstrol dimethyl ether remains unchanged when heated alone or in an atmosphere of sulphur dioxide or hydrogen chloride, whereas when heated to 250° in presence of 1% of iodine it is resinified. Traces of hexœstrol dimethyl ether are obtained when *iso*hexœstrol dimethyl ether is heated to 300—320° in presence of palladium–charcoal, and when heated at 300° with sulphur some isomerisation occurs but much of the material is resinified. When the *iso*-ether is heated at 300° in an atmosphere of hydrogen sulphide, it is partially isomerised to hexœstrol dimethyl ether, the yield being 50% when allowance is made for the recovered *iso*-ether. In the same way, hexœstrol dimethyl ether is partially isomerised to *iso*hexœstrol dimethyl ether, so the isomerisation is reversible. *iso*Hexœstrol remains unchanged when heated at 250° with pyridine–piperidine, is recovered unchanged, after hydrolysis, by heating with acetic anhydride at 250°, and is completely decomposed on heating with hydrogen sulphide at 300°.

These isomerisations provide a further addition to the small number of examples in which an interconversion of optical isomerides has been observed with compounds which are incapable of conversion into an unsaturated isomeride, for example, by enolisation of a carbonyl group adjacent to an asymmetric carbon atom. It is difficult to envisage the observed isomerisations except on the basis either of reversible dehydrogenation or of fission and recombination.

### EXPERIMENTAL.

*Isomerisation of Hexœstrol Dimethyl Ether.*—Undried hydrogen sulphide was slowly passed through *iso*hexœstrol dimethyl ether (50 g.; m. p. 53—54°) at 305—310° (oil-bath) for 3 hours. The small distillate, which contained anethole and some hexœstrol dimethyl ether, was combined with the pale red residue, dissolved in light petroleum (150 c.c., b. p. 60—80°) and cooled to 0° overnight. The crystals were collected, washed with a little light petroleum, and recrystallised from boiling alcohol (150 c.c.). The dimethyl ether (5·1 g.) so obtained melted at 143·5—144°, undepressed by authentic hexœstrol dimethyl ether, and afforded hexœstrol, m. p. and mixed m. p. 185°, on demethylation with alcoholic potassium hydroxide. Evaporation of the mother-liquors and distillation of the residue afforded anethole (2·5 g.) and a fraction (40·3 g.), b. p. 184—187°/3 mm., which was practically pure *iso*hexœstrol dimethyl ether.

*Isomerisation of* iso*Hexœstrol Dimethyl Ether.*—Hexœstrol dimethyl ether (10 g.; m. p. 144°) was heated at 305—310° for 3 hours in a slow stream of undried hydrogen sulphide. The residue, which partly crystallised on cooling, was dissolved in boiling light petroleum (100 c.c.; b. p. 60—80°), cooled, and filtered from hexœstrol dimethyl ether; a further crop was obtained by concentrating the filtrate to 10 c.c. (total yield, 5·7 g.). The rest of the solvent was removed, and the residue distilled, giving (1) a fraction (1·2 g.) below 105°/20 mm., consisting mainly of anethole, and (2) a portion (1·2 g.), b. p. 215—225°/20 mm. Fraction (2) was dissolved in alcohol (6 c.c.), filtered from a small amount of hexœstrol dimethyl ether, and cooled to 0°. The crystals (0·45 g.) so obtained melted at 53—54° either alone or after admixture with authentic *iso*hexœstrol dimethyl ether.

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NOTTINGHAM. [*Received, February 23rd,* 1943.]

# 58. *Derivatives of* 1 : 2 : 4 : 5-*Tetrachlorobenzene. Part I. Nitro- and Amino-compounds.*

By A. T. Peters, F. M. Rowe, and D. M. Stead.

2 : 3 : 5 : 6-*Tetrachloro*-4-*nitroaniline* (IV), prepared from 2 : 3 : 5 : 6-tetrachloro-1 : 4-dinitrobenzene (III) by reduction with sodium hydrosulphite or by amination with alcoholic ammonia, is diazotisable with nitrosylsulphuric acid at 60—70° and then couples to form azo-dyes. 2 : 3 : 5 : 6-Tetrachloro-aniline, -1 : 4-diaminobenzene, and -4-aminoanisole were also examined as intermediates for azo-dyes. 2 : 3 : 5 : 6-*Tetrachloro*-4-*nitrophenol* (V), best prepared by nitrating 2 : 3 : 5 : 6-tetrachlorophenol, was also a by-product of the preparation of (IV) and 2 : 3 : 5 : 6-tetrachloro-4-nitroanisole from (III) by amination and the action of sodium methoxide, respectively. Replacement of chlorine or demethylation occurred when 2 : 3 : 5 : 6-*tetrachlorobenzenediazonium sulphate* or its 4-methoxy-derivative was decomposed in aqueous sodium acetate, giving 3 : 4 : 6-*trichlorobenzene*-2-*diazo*-1-*oxide* or 2 : 3 : 5 : 6-*tetrachlorobenzene*-4-*diazo*-1-*oxide*, respectively.

We have studied the chemistry of 1 : 2 : 4 : 5-tetrachlorobenzene with special reference to derivatives which may be used as intermediates for azo-dyes. The preparation of 2 : 3 : 5 : 6-tetrachloroaniline (I) (Holleman, *Rec. Trav. chim.*, 1920, **39**, 736; Dyson, George, and Hunter, J., 1926, 304) has been improved. Compound (I) was diazotised satisfactorily with nitrosylsulphuric acid at 60° and then coupled normally to form azo-dyes. On the other hand, when the diazonium solution from (I) was kept in aqueous sodium acetate at room temperature for several days, partial replacement of the 2-chloro-atom occurred with formation of 3 : 4 : 6-*trichlorobenzene*-2-*diazo*-1-*oxide*, identical with that obtained from 3 : 4 : 6-trichloro-*o*-anisidine (see Harrison, Peters, and Rowe, following paper).

2 : 3 : 5 : 6-Tetrachloro-1 : 4-diaminobenzene (II), prepared by Krause (*Ber.*, 1879, **12**, 51) from 1 : 4-dichlorodi-iminobenzoquinone and fuming hydrochloric acid, was readily obtained by reducing 2 : 3 : 5 : 6-tetrachloro-1 : 4-dinitrobenzene (III) with tin and hydrochloric acid in alcohol, and formed only *mono-* and *tetra-acetyl* derivatives. Diazotisation of the monoacetyl compound with nitrosylsulphuric acid led to hydrolysis of the acetyl group, so that, on coupling with alkaline β-naphthol, 2 : 3 : 5 : 6-*tetrachloro*-4-*aminobenzeneazo*-β-*naphthol* was obtained.

2 : 3 : 5 : 6-*Tetrachloro*-4-*nitroaniline* (IV) was prepared conveniently by reducing compound (III) with aqueous-alcoholic sodium hydrosulphite, and, owing to the liability of the 1-nitro-group in (III), was also obtained equally well by aminating it with alcoholic ammonia at 110—120°, but nitrating (I) gave (IV) in much lower yield. The nitroamine (IV) gave a *mono-* and a *di-acetyl* derivative, the latter being reduced to 2 : 3 : 5 : 6-*tetrachloro*-4-*aminodiacetanilide*, which could not be obtained directly from (II), and converted by further acetylation into 2 : 3 : 5 : 6-tetrachloro-1 : 4-tetra-acetyldiaminobenzene. Diazotisation of (IV) required the use of nitrosylsulphuric acid at 60—70° and azo-compounds were prepared from the resulting solution.

Nitration of 2 : 3 : 5 : 6-tetrachloroanisole gave 2 : 3 : 5 : 6-tetrachloro-4-nitroanisole in excellent yield and, although, owing to the lability of the 1-nitro-group in compound (III), it was also readily obtained from (III) and sodium methoxide, a little 2 : 3 : 5 : 6-*tetrachloro*-4-*nitrophenol* (V) was formed at the same time in the latter case. Compound (V) was also obtained as a by-product during amination of (III), but was best prepared by nitrating 2 : 3 : 5 : 6-tetrachlorophenol (cf. Holleman, *loc. cit.*). 2 : 3 : 5 : 6-Tetrachloro-4-aminoanisole (cf. Bureš and Hutter, *Časopis Českoslov. Lék.*, 1931, **11**, 29), readily obtained by reducing the corresponding nitro-compound with aqueous-alcoholic sodium hydrosulphite, was diazotisable with warm nitrosylsulphuric acid and then coupled with β-naphthol in acetic acid to form 2 : 3 : 5 : 6-*tetrachloro*-4-*methoxybenzeneazo*-β-*naphthol*. On the other hand, 2 : 3 : 5 : 6-tetrachloro-4-methoxybenzenediazonium sulphate was readily convertible at room temperature into 2 : 3 : 5 : 6-*tetrachlorobenzene*-4-*diazo*-1-*oxide* (VI), which coupled with alkaline β-naphthol to form 2 : 3 : 5 : 6-*tetrachloro*-4-*hydroxybenzeneazo*-β-*naphthol* (VII). The azo-dye (VII) was also obtained when the crude diazo-oxide from 2 : 3 : 5 : 6-tetrachloro-4-nitrobenzenediazonium sulphate and aqueous sodium acetate was treated with alkaline β-naphthol, indicating the replacement of the 4-nitro-group and not the 2-chloro-atom with formation of some (VI) in the latter case.

2 : 3 : 5 : 6-*Tetrachloro*-, 2 : 3 : 5 : 6-*tetrachloro*-4-*nitro*-, and 2 : 3 : 5 : 6-*tetrachloro*-4-*methoxy*-2′-*hydroxy*-3′-*naphthanilide* were best prepared from the respective amines and 2-hydroxy-3-naphthoyl chloride in nitrobenzene.

## Experimental.

Microanalyses were carried out by Dr. G. Weiler and Dr. F. B. Strauss, of Oxford.

2 : 3 : 5 : 6-*Tetrachloroaniline* (I) *and its Derivatives* (Lesimple, *Z. Chem.*, 1868, 227; cf. Holleman; Dyson, George, and Hunter, *locc. cit.*).—2 : 3 : 5 : 6-Tetrachloronitrobenzene (39 g.), m. p. 99·5—100·5°, was added during 30 minutes to a stirred, boiling mixture of alcohol (300 c.c.), hydrochloric acid (6 c.c.) and iron powder (40 g.). After refluxing and stirring for 6 hours, excess of sodium carbonate was added, the mixture filtered hot, and the filtrate diluted with water. The precipitate was collected, boiled with hydrochloric acid, washed with water, and crystallised from alcohol, forming long colourless needles, m. p. 107—108° (yield, 32·5 g.; 94·2%) (Dyson, George, and Hunter, *loc. cit.*, gave m. p. 90°). 2 : 3 : 5 : 6-Tetrachloroaniline forms an unstable hydrochloride with dry hydrogen chloride in benzene, but is insoluble in concentrated hydrochloric acid. The base (46·2 g.) was refluxed with acetic acid (100 c.c.) and acetic anhydride (20 g.) for 24 hours to give the *monoacetyl* derivative, which crystallised from alcohol in lustrous, colourless needles, m. p. 213—214° (yield, 39·6 g.; 72·5%) (Found: C, 35·35; H, 1·8; N, 4·7; Cl, 52·4. $C_8H_5ONCl_4$ requires C, 35·2; H, 1·8; N, 5·1; Cl, 52·0%). On refluxing the base (2 g.) with acetic anhydride (10 c.c.) or acetyl chloride (50 c.c.) for 8 hours, the *diacetyl* derivative was obtained, which crystallised from alcohol in long, colourless needles, m. p. 175—176° (yield,

2·4 g., 88·2%, or 2·6 g., 95·6%, respectively) (Found : C, 38·45; H, 2·3; N, 4·9; Cl, 45·4. $C_{10}H_7O_2NCl_4$ requires C, 38·1; H, 2·2; N, 4·4; Cl, 45·1%). 2 : 3 : 5 : 6-Tetrachlorobenzenediazonium sulphate can be isolated as a cream powder by diazotising the base in sulphuric acid and precipitating the product with alcohol at −3°; on solution in water and addition of a 50% solution of zinc chloride in hydrochloric acid at 0°, yellow needles of the *zinc chloride* double salt separated (Found : 64% of diazonium salt by coupling with β-naphthol. $C_6HCl_4N_2Cl,ZnCl_2$ requires 67·1%). 2 : 3 : 5 : 6-Tetrachloroaniline (1·1 g.) was diazotised with nitrosylsulphuric acid (prepared from 0·35 g. of sodium nitrite and 3 c.c. of sulphuric acid) at 60° and coupled with alkaline β-naphthol; 2 : 3 : 5 : 6-*tetrachlorobenzeneazo-β-naphthol* crystallised from toluene or acetic acid in fine, red needles, m. p. 212° (yield, 1·7 g.; 93%) (Found : N, 7·4. $C_{16}H_8ON_2Cl_4$ requires N, 7·25%).

2 : 3 : 5 : 6-*Tetrachloro-2′-hydroxy-3′-naphthanilide.*—2 : 3 : 5 : 6-Tetrachloroaniline (7·7 g.), 2-hydroxy-3-naphthoyl chloride (7 g.), and nitrobenzene (20 c.c.) were refluxed for 2 hours. Excess of sodium carbonate was added, and the mixture distilled with steam. The residue was extracted with 10% sodium hydroxide solution, the extract acidified, and the precipitate crystallised from acetic acid, forming brownish-yellow, irregular prisms, m. p. 232° (yield, 8·5 g.; 64·6%) (Found : C, 50·6; H, 2·55; Cl, 34·15. $C_{17}H_9O_2NCl_4$ requires C, 50·9; H, 2·25; Cl, 35·4%).

3 : 4 : 6-*Trichlorobenzene-2-diazo-1-oxide.*—2 : 3 : 5 : 6-Tetrachloroaniline (2·3 g.) was diazotised, and the solution poured on ice (200 g.) and sodium acetate (16 g.); after 4 days, the precipitate was collected and crystallised from dilute sulphuric acid, forming orange-yellow needles, m. p. 117—118° (decomp.) (yield, 0·56 g.; 25%). The diazo-oxide was identical with that prepared from 3 : 4 : 6-trichloro-*o*-anisidine (see Harrison, Peters, and Rowe, *loc. cit.*).

2 : 3 : 5 : 6-*Tetrachloro*-1 : 4-*diaminobenzene* (II) *and its Derivatives.*—(*a*) 2 : 3 : 5 : 6-Tetrachloro-1 : 4-dinitrobenzene (5 g.), m. p. 232° with sublimation (prepared in 60% yield by the method of Berckmans and Holleman, *Rec. Trav. chim.*, 1925, **44**, 851, but the monohydrate being used in place of 20% oleum), was refluxed with tin (10 g.), alcohol (150 c.c.), and hydrochloric acid (50 c.c.) for 1 hour, and the crystals which separated on cooling were ground with aqueous sodium hydroxide. The diamine crystallised from toluene in lustrous, colourless needles, m. p. 222—223° (yield, 3·4 g.; 85%) (Found : C, 29·85; H, 1·6; N, 11·5. Calc. for $C_6H_4N_2Cl_4$ : C, 29·3; H, 1·6; N, 11·4%). (*b*) Compound (III) (3 g.) was refluxed with alcohol (200 c.c.) and sodium hydrosulphite (12 g.), water (100 c.c.) being gradually added and boiling continued until the solution was colourless. After filtration, the diamine was precipitated from the filtrate with water (yield, 2 g.; 81%).

The diamine (2 g.) was boiled with acetic anhydride (10 c.c.) for 3 minutes; the *monoacetyl* derivative crystallised from acetic acid in small, colourless rhombohedra, m. p. 276° (yield, 2·3 g.; 100%) (Found : C, 33·7; H, 2·2; N, 9·75. $C_8H_6ON_2Cl_4$ requires C, 33·3; H, 2·1; N, 9·7%). Refluxing (II) (4 g.) with acetic anhydride (20 c.c.) for 20 hours gave the *tetra-acetyl* derivative, which separated from acetic acid in large, colourless prisms, m. p. 205—209° (yield, 6 g.; 89·4%) (Found : C, 40·3; H, 2·85; N, 7·05; Cl, 34·4. $C_{14}H_{12}O_4N_2Cl_4$ requires C, 40·6; H, 2·9; N, 6·7; Cl, 34·3%).

The monoacetyl derivative (7·2 g.) was warmed on the water-bath with nitrosylsulphuric acid for 1 hour. The diazonium solution coupled with alkaline β-naphthol to give 2 : 3 : 5 : 6-*tetrachloro-4-aminobenzeneazo-β-naphthol*, which crystallised from toluene in deep red needles, m. p. 257—258° (decomp.) (yield, 6 g.; 60%) (Found : C, 47·9; H, 2·25; Cl, 35·4. $C_{16}H_9ON_3Cl_4$ requires C, 47·9; H, 2·2; Cl, 35·4%).

2 : 3 : 5 : 6-*Tetrachloro-4-nitroaniline* (IV) *and its Derivatives.*—(*a*) Sodium hydrosulphite (5·5 g.) in water (50 c.c.) was added to 2 : 3 : 5 : 6-tetrachloro-1 : 4-dinitrobenzene (3 g.) in boiling alcohol (180 c.c.) and, after refluxing for 20 minutes, the mixture was cooled. The precipitate crystallised from alcohol in fine, yellow needles, m. p. 216—217°, turning brown on the surface in air (yield, 1·8 g.; 65·2%) (Found : C, 26·8; H, 1·0; Cl, 51·8. $C_6H_2O_2N_2Cl_4$ requires C, 26·1; H, 0·7; Cl, 51·5%). (*b*) 2 : 3 : 5 : 6-Tetrachloro-1 : 4-dinitrobenzene (1·7 g.) was heated with 2·8N-alcoholic ammonia (8 c.c.) at 110—120° for 8 hours in a sealed tube, and the product precipitated by water (yield, 1 g.; 65·4%).

2 : 3 : 5 : 6-*Tetrachloro-4-nitroaniline* is insoluble in hydrochloric acid and does not form a hydrochloride with hydrogen chloride in chloroform. Heating it (5·5 g.) with acetyl chloride (2 c.c.) in toluene (20 c.c.) at 110—120° for 3 hours in a sealed tube gave the *monoacetyl* derivative, which crystallised from alcohol in fine, colourless needles, m. p. 252—253° (yield, 4·4 g.; 69·1%) (Found : C, 30·3; H, 1·3; N, 8·65; Cl, 45·0. $C_8H_4O_3N_2Cl_4$ requires C, 30·2; H, 1·3; N, 8·8; Cl, 44·65%). The *diacetyl* derivative, obtained when (IV) (5 g.) was refluxed with acetic anhydride (20 c.c.) and a drop of sulphuric acid, crystallised from alcohol in fern-like clusters of colourless needles, m. p. 168—169° (yield, 6·1 g.; 93·5%) (Found : C, 33·5; H, 1·7; N, 7·4; Cl, 40·0. $C_{10}H_6O_4N_2Cl_4$ requires C, 33·3; H, 1·7; N, 7·7; Cl, 39·4%). Reduction of the diacetyl derivative (2·4 g.) by refluxing with sodium hydrosulphite (5 g.), water (25 c.c.), and alcohol (55 c.c.) for 15 minutes, followed by precipitation with water, gave 2 : 3 : 5 : 6-*tetrachloro-4-aminodiacetanilide*, which crystallised from aqueous alcohol in long, colourless, rectangular plates, m. p. 194—195° (yield, 1·6 g.; 72·8%) (Found : C, 36·55; H, 2·7; N, 8·4; Cl, 42·8. $C_{10}H_8O_2N_2Cl_4$ requires C, 36·4; H, 2·4; N, 8·5; Cl, 43·0%), converted by boiling acetic anhydride and a little sulphuric acid into 2 : 3 : 5 : 6-tetrachloro-1 : 4-tetra-acetyldiaminobenzene, m. p. and mixed m. p. 205—209°.

2 : 3 : 5 : 6-Tetrachloro-4-nitroaniline (5·5 g.), diazotised with nitrosylsulphuric acid at 60—70°, was added to an aqueous solution of excess of sodium β-naphthol-1-sulphonate, the precipitated diazosulphonate ground with sodium carbonate solution, and the mixture acidified. 2 : 3 : 5 : 6-*Tetrachloro-4-nitrobenzeneazo-β-naphthol* crystallised from toluene in small red needles, with a bronze lustre, m. p. 282—284° (decomp.) (yield, 6 g.; 70%) (Found : N, 9·7; Cl, 33·2. $C_{16}H_7O_3N_3Cl_4$ requires N, 9·7; Cl, 32·9%). 2 : 3 : 5 : 6-Tetrachloro-4-nitroaniline (5·5 g.) was diazotised and coupled with 2-hydroxy-3-naphthanilide in acetic acid at 70°; 2 : 3 : 5 : 6-*tetrachloro-4-nitrobenzeneazo-2′-hydroxy-3′-naphthanilide* crystallised from acetic anhydride in small, red needles, m. p. 296° (decomp.) (yield, 6·6 g.; 60%) (Found : N, 10·2; Cl, 25·7. $C_{22}H_{12}O_4N_4Cl_4$ requires N, 10·2; Cl, 25·8%).

2 : 3 : 5 : 6-*Tetrachloro-4-nitro-2′-hydroxy-3′-naphthanilide*, prepared similarly to the unnitrated analogue, crystallised from acetic acid in faintly yellow plates, m. p. 269—270° (yield, 40%) (Found : C, 45·8; H, 2·0; Cl, 31·7. $C_{17}H_8O_4N_2Cl_4$ requires C, 45·7; H, 1·8; Cl, 31·8%).

2 : 3 : 5 : 6-*Tetrachloro-4-nitroanisole* (cf. Berckmans and Holleman, *loc. cit.*).—(*a*) 2 : 3 : 5 : 6-Tetrachloroanisole (4 g.) was added to nitric acid (*d* 1·5, 12 c.c.) at 0°. The mixture was poured on ice, and the precipitate crystallised from aqueous alcohol, forming long, colourless needles, m. p. 112—113° (yield, 4·5 g.; 95%) (Berckmans and Holleman gave m. p. 105—106°). (*b*) 2 : 3 : 5 : 6-Tetrachloro-1 : 4-dinitrobenzene (15·3 g.) was heated with 0·2N-sodium methoxide (250 c.c.) on the water-bath for 30 minutes, cooled, and diluted with water (yield, 13·4 g.; 92·1%). After the 2 : 3 : 5 : 6-tetrachloro-4-nitroanisole had been collected, the filtrate was evaporated to a small bulk and acidified with nitric acid; a little 2 : 3 : 5 : 6-tetrachloro-4-nitrophenol, m. p. and mixed m. p. 148—149° (decomp.), was then obtained.

2 : 3 : 5 : 6-*Tetrachloro-4-aminoanisole and its Derivatives* (cf. Bureš and Hutter, *loc. cit.*).—2 : 3 : 5 : 6-Tetrachloro-4-nitroanisole (14·5 g.) was refluxed with sodium hydrosulphite (50 g.), alcohol (400 c.c.), and water (200 c.c.) for 30 minutes; after concentration and cooling, the amine separated and was crystallised from alcohol, forming faintly pink, long needles, m. p. 107—108° (yield, 12·5 g.; 96·1%) (diacetyl derivatives, m. p. 105—106°; Bureš and Hutter gave m. p. 101°). The base (4 g.) was diazotised in warm nitrosylsulphuric acid and coupled with β-naphthol in acetic acid; 2 : 3 : 5 : 6-*tetrachloro-4-methoxybenzeneazo-β-naphthol* crystallised from acetic acid in clusters of red needles, m. p. 204—205° (yield, 5·2 g.; 91·2%) (Found : N, 7·3; Cl, 34·2. $C_{17}H_{10}O_2N_2Cl_4$ requires N, 6·7; Cl, 34·1%). 2 : 3 : 5 : 6-*Tetra-*

*chloro-4-methoxy-2'-hydroxy-3'-naphthanilide* crystallised from acetic acid in colourless rhombs or needles, m. p. 208° (Found: C, 50·4; H, 2·65. $C_{19}H_{11}O_3NCl_4$ requires C, 50·1; H, 2·55%).

2 : 3 : 5 : 6-*Tetrachloro-4-nitrophenol* (V).—2 : 3 : 5 : 6-Tetrachlorophenol (2·3 g.) was added to nitric acid (*d* 1·5; 8 c.c.) and acetic acid (40 c.c.) at 10° and, after 1 hour, the mixture was poured on ice. The precipitate crystallised from aqueous acetic acid in colourless needles, m. p. 148—149° (decomp.) (yield, 1·8 g.; 65%) (Found: C, 26·5; H, 0·65; N, 5·1; Cl, 51·1. $C_6HO_3NCl_4$ requires C, 26·0; H, 0·4; N, 5·0; Cl, 51·2%). Concentration of the filtrate obtained after separation of 2 : 3 : 5 : 6-tetrachloro-4-nitroaniline prepared by amination of 2 : 3 : 5 : 6-tetrachloro-1 : 4-dinitrobenzene (see p. 234), followed by extraction with benzene, gave 2 : 3 : 5 : 6-tetrachloro-4-nitrophenol (yield, 5%), m. p. and mixed m. p. 148—149° (decomp.), and the latter was also isolated in small quantity as a by-product in the preparation of 2 : 3 : 5 : 6-tetrachloro-4-nitroanisole by method (*b*) (*q.v.*). The *acetate*, prepared by boiling 2 : 3 : 5 : 6-tetrachloro-4-nitrophenol (0·3 g.) with acetic anhydride (2 c.c.) and a drop of sulphuric acid for 5 minutes, crystallised from aqueous alcohol in long, colourless needles, m. p. 113—114° (yield, 0·3 g.; 87·5%) (Found: C, 30·7; H, 1·4; Cl, 44·45. $C_8H_3O_4NCl_4$ requires C, 30·1; H, 0·9; Cl, 44·5%).

2 : 3 : 5 : 6-*Tetrachlorobenzene-4-diazo-1-oxide* (VI).—2 : 3 : 5 : 6-Tetrachloro-4-aminoanisole (4 g.) was diazotised with warm nitrosylsulphuric acid and diluted (500 c.c.) with addition of sodium acetate; after 4 hours, the *diazo-oxide* was collected and crystallised from ligroin, forming yellow needles, darkening at 120° and exploding at 131° (yield, 3·3 g.; 83%) (Found: N, 11·45; Cl, 55·1. $C_6ON_2Cl_4$ requires N, 10·85; Cl, 55·0%). On boiling the diazo-oxide (4 g.) with acetic anhydride (45 c.c.) for 1 minute, colourless needles of 2 : 3 : 5 : 6-tetrachloro-1 : 4-diacetoxybenzene separated, which were recrystallised from acetic acid, forming colourless needles, m. p. 251° (with sublimation) (yield, 1·6 g.; 33·6%) (Found: C, 36·4; H, 1·9; Cl, 42·4. Calc. for $C_{10}H_6O_4Cl_4$: C, 36·1; H, 1·8; Cl, 42·8%), not depressed on admixture with the product obtained by acetylation of tetrachloroquinol (Graebe, *Annalen*, 1891, **263**, 29). On coupling the diazo-oxide with β-naphthol in 1% sodium hydroxide solution, a brown solution was obtained; acidification with acetic acid gave 2 : 3 : 5 : 6-*tetrachloro-4-hydroxybenzeneazo-β-naphthol* (VII), which crystallised from toluene in fine, red needles, m. p. 264—265° (decomp.) (Found: C, 47·95; H, 2·2; N, 7·2; Cl, 35·3. $C_{16}H_8O_2N_2Cl_4$ requires C, 47·8; H, 2·0; N, 7·0; Cl 35·3%). Compound (VII) was also formed when diazotised 2 : 3 : 5 : 6-tetrachloro-4-nitroaniline was added to aqueous sodium acetate at 0° and, after 1 hour, the resulting yellow precipitate of crude diazo-oxide coupled with alkaline β-naphthol.

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## 59. *Polyhalogeno-o-anisidines and their Derivatives.*

By W. S. W. HARRISON, A. T. PETERS, and F. M. ROWE.

3 : 4 : 6-*Trichloro-2-nitroanisole* (IV), best prepared by nitrating 2 : 4 : 5-trichloroanisole (III), was reduced to 3 : 4 : 6-*trichloro-o-anisidine* (V), which with nitric acid (*d* 1·5) gave 2 : 3 : 5-*trichloro-4-nitro-6-methoxy-N-nitroaniline* (VI), converted by nitrous acid into the 1-diazonium compound or by acetic acid into 2 : 3 : 5-*trichloro-6-methoxy-p-benzoquinone*. Decomposition of 2 : 3 : 5-trichloro-6-methoxybenzenediazonium sulphate in presence of aqueous sodium acetate was accompanied by demethylation and gave 3 : 4 : 6-*trichlorobenzene-2-diazo-1-oxide*. Nitration of either *mono-* or *di-acetyl-3 : 4 : 6-trichloro-o-anisidine* gave *monoacetyl-3 : 4 : 6-trichloro-5-nitro-o-anisidine*, from which 3 : 4 : 6-*trichloro-5-nitro-o-anisidine* (VII) was obtained; the latter is convertible into (VI). Other compounds prepared include 3 : 4 : 6-*trichloro-5-amino-2-acetyl-* or *-diacetyl-aminoanisole*, 3 : 4 : 6-*trichloro-2 : 5-diaminoanisole*, and 3 : 4 : 6-*trichloro-5-bromo-o-anisidine*.

THE object of this work was to prepare tri- and tetra-chloro-*o*-anisidines which may be used as intermediates for azo-dyes. By the action of sodium methoxide on 1 : 2 : 4 : 5-tetrachlorobenzene (I), Holleman (*Rec. Trav. chim.*, 1920, **39**, 736) obtained 2 : 4 : 5-trichlorophenol (II) in addition to 2 : 4 : 5-trichloroanisole (III), but we prepared (II) as the sole product by heating (I) with methyl-alcoholic sodium hydroxide at 160° (cf. I.G. Farb.; D.R.-P. 349,794) and (III) is best prepared by methylating (II). Methylation of 3 : 4 : 6-trichloro-2-nitrophenol (Kohn and Fink, *Monatsh.*, 1931, **58**, 73) with methyl sulphate in xylene–potassium carbonate at 140°, or with methyl toluene-*p*-sulphonate, gave only 10% yields of 3 : 4 : 6-*trichloro-2-nitroanisole* (IV), which was best prepared (66% yield) by nitrating (III). 3 : 4 : 6-*Trichloro-o-anisidine* (V), prepared by reducing (IV) with iron, alcohol, and aqueous acetic acid, was treated with nitric acid (*d* 1·5) in acetic acid below 10° and gave 2 : 3 : 5-*trichloro-4-nitro-6-methoxy-N-nitroaniline* (VI). It was converted into the 1-diazonium compound by nitrous acid, whereas refluxing (VI) with acetic acid gave 2 : 3 : 5-*trichloro-6-methoxy-p-benzoquinone*. Compound (V) was best diazotised with warm nitrosylsulphuric acid; on coupling with β-naphthol in sodium hydroxide or acetic acid solution, 2 : 3 : 5-*trichloro-6-methoxybenzeneazo-β-naphthol* was formed. Decomposition of the diazonium sulphate or nitrite derived from (V) resulted in demethylation and gave 3 : 4 : 6-*trichlorobenzene-2-diazo-1-oxide*, also obtained from 2 : 3 : 5 : 6-tetrachlorobenzenediazonium sulphate (see Peters, Rowe, and Stead, preceding paper), and convertible into (II) by heating with alcohol at 150°. On adding the diazo-oxide to alkaline β-naphthol, a deep red solution (disodium salt) was formed; neutralisation gave a blue solution (monosodium salt), and acidification the insoluble red 2 : 3 : 5-*trichloro-6-hydroxybenzeneazo-β-naphthol*. It is unusual for the decomposition of the diazonium salt of an *o*-anisidine to be accompanied by demethylation. We found that decomposition of the diazonium salt of 4-chloro-3 : 5-dibromo-*o*-anisidine is also accompanied by demethylation, giving 4-chloro-3 : 5-dibromobenzene-2-diazo-1-oxide, so that halogen in position 6 is not essential for such demethylation to occur.

Nitration of either *mono-* or *di-acetyl-3 : 4 : 6-trichloro-o-anisidine* gave *monoacetyl-3 : 4 : 6-trichloro-5-nitro-o-anisidine*, hydrolysed by acid to 3 : 4 : 6-*trichloro-5-nitro-o-anisidine* (VII), which is convertible into (VI). It is probable that, during the nitration of the above diacetyl compound, one acetyl group is replaced by a

nitro-group with formation of the *N*-nitro-*N*-acetyl derivative, followed by migration of the nitro-group to position 5. That the methoxy-group is removed in preparing 3 : 4 : 6-trichloro-5-nitrobenzene-2-diazo-1-oxide from (VII) was proved by coupling with 2-hydroxy-3-naphthanilide, 2 : 3 : 5-*trichloro*-4-*nitro*-6-*hydroxybenzeneazo*-2′-*hydroxy*-3′-*naphthanilide* (VIII) being formed. Diazotised 3 : 4 : 6-trichloro-5-nitro-*o*-anisidine with the same coupling component in acetic acid, or even in a mixture of acetic and sulphuric acids, gave 2 : 3 : 5-*trichloro*-4-*nitro*-6-*methoxybenzeneazo*-2′-*hydroxy*-3′-*naphthanilide*, together with a little (VIII).

3 : 4 : 6-Trichloro-5-nitro-*o*-anisidine (VII) was reduced with iron in alcohol and acetic acid to 3 : 4 : 6-*trichloro*-2 : 5-*diaminoanisole*, convertible into the 2 : 5-*bisacetamido*-derivative. 3 : 4 : 6-*Trichloro*-5-*amino*-2-*acetyl*- (IX) and -2-*diacetyl*-*aminoanisole* were obtained by reducing the mono- and di-acetyl derivative of compound (VII), respectively, and diazotisation of (IX) did not require the use of nitrosylsulphuric acid.

3 : 4 : 6-*Trichloro*-5-*bromo*-*o*-*anisidine* was readily prepared by brominating (V); it was diazotised with nitrosylsulphuric acid and then coupled normally to form azo-dyes. On the other hand, when the diazonium solution was kept for 1 day in presence of sodium acetate, the 2-diazo-1-oxide separated, and its constitution was proved by conversion into 2 : 3 : 5-*trichloro*-4-*bromo*-6-*hydroxybenzeneazo*-2′-*hydroxy*-3′-*naphthanilide*.

Bureš and Havlinova (*Časopis Českoslov. Lék.*, 1929, **9**, 101, 129, 153) obtained 3 : 4 : 5 : 6-tetrachloro-*o*-anisidine by chlorinating acet-*o*-anisidide in acetic acid, followed by hydrolysis, but we prepared it readily by chlorinating 3 : 4 : 6-trichloro-*o*-anisidine in chloroform and diazotised it with nitrosylsulphuric acid.

## Experimental.

Microanalyses were carried out by Dr. G. Weiler and Dr. F. B. Strauss, of Oxford.

2 : 4 : 5-*Trichloro*-*phenol* (II) *and* -*anisole* (III).—1 : 2 : 4 : 5-Tetrachlorobenzene (31 g.), sodium hydroxide (22·6 g.), and methyl alcohol (100 c.c.) were heated in an autoclave at 160° for 7 hours. After dilution to 500 c.c. with water, the mixture was acidified and distilled with steam. The distillate was cooled in ice and the 2 : 4 : 5-trichlorophenol, m. p. 63°, collected (yield, 24·2 g.; 84·9%). It crystallised from ligroin in colourless, feathery needles, m. p. 66°, b. p. 244—248°/746 mm. (cf. I.G. Farb., *loc. cit.*).

Methyl sulphate (9 c.c.) was added gradually to compound (II) (5 g.) in 20% aqueous sodium hydroxide (30 c.c.), the mixture refluxed for 3 hours and poured into water, and the product collected. 2 : 4 : 5-Trichloroanisole crystallised from alcohol in colourless needles, m. p. 75° (yield, 4·2 g.; 78·5%).

3 : 4 : 6-*Trichloro*-2-*nitrophenol*.—Nitric acid (*d* 1·43; 3·6 c.c.) in acetic acid (7 c.c.) was added to 2 : 4 : 5-trichlorophenol (6 g.) in acetic acid (7 c.c.) at <10°; after 30 minutes, the mixture, containing crystals, was added to ice, and the precipitate filtered off and crystallised from ligroin, forming palé yellow leaflets, m. p. 92—93° (Kohn and Fink, *loc. cit.*, recorded m. p. 81°) (yield, 6·8 g.; 90%).

3 : 4 : 6-*Trichloro*-2-*nitroanisole* (IV).—2 : 4 : 5-Trichloroanisole (30 g.) was added in small amounts to nitric acid (*d* 1·5; 80 c.c.) at 5—10°; after stirring for 40 minutes, the mixture was poured on ice (500 g.), and the product collected, dissolved in warm alcohol, and stirred at 0°; 3 : 4 : 6-*trichloro*-2-*nitroanisole* then crystallised in colourless needles, m. p. 19—21°; b. p. 288° (yield, 24·3 g.; 66·6%) (Found : C, 33·6; H, 1·5; N, 5·7; Cl, 41·4. $C_7H_4O_3NCl_3$ requires C, 32·8; H, 1·5; N, 5·5; Cl, 41·5%).

3 : 4 : 6-*Trichloro*-*o*-*anisidine* (V).—Iron powder (30 g.) was added gradually to the nitro-compound (IV) (20 g.) in acetic acid (50 c.c.), alcohol (60 c.c.), and water (40 c.c.), and, after refluxing for 1 hour, the mixture was filtered on ice; the precipitate crystallised from ligroin in almost colourless needles, m. p. 61—62° (yield, 14·1 g.; 79·8%) (Found : C, 36·8; H, 2·7; N, 6·6; Cl, 46·7. $C_7H_6ONCl_3$ requires C, 37·1; H, 2·65; N, 6·2; Cl, 47·0%). The base is readily soluble in most organic solvents, but is insoluble in concentrated hydrochloric acid, and is sparingly volatile with steam. The *monoacetyl* derivative separated after (V) (5 g.) had been refluxed with acetyl chloride (1·8 c.c.) in toluene (60 c.c.) for 1 hour and cooled; it crystallised from aqueous alcohol in colourless needles, m. p. 181—182° (yield, 4·5 g.; 76%) (Found : C, 40·7; H, 3·0; N, 5·0; Cl, 40·0. $C_9H_8O_2NCl_3$ requires C, 40·2; H, 3·0; N, 5·2; Cl, 39·7%). The *diacetyl* derivative, prepared by refluxing the base (V) (5 g.) with acetic anhydride (25 c.c.) and a drop of pyridine for 3 hours, crystallised from aqueous alcohol in colourless needles, m. p. 128—129° (yield, 5·8 g.; 84·5%) (Found : C, 43·2; H, 3·1; Cl, 34·4. $C_{11}H_{10}O_3NCl_3$ requires C, 42·7; H, 3·2; Cl, 34·3%).

3 : 4 : 6-*Trichloro*-5-*nitro*-*o*-*anisidine* (VII).—Mono- (7 g.) or di-acetyl-3 : 4 : 6-trichloro-*o*-anisidine (7 g.) was added to nitric acid (*d* 1·5; 30 c.c.) at <10° and, after 30 minutes, the mixture was poured on ice; the *monoacetyl*-3 : 4 : 6-*trichloro*-5-*nitro*-*o*-*anisidine* crystallised from aqueous alcohol in colourless needles, m. p. 237° (yield, 7 g., 86%, or 5·5 g., 77·7%, respectively) (Found : C, 34·7; H, 2·3; N, 9·0; Cl, 33·9. $C_9H_7O_4N_2Cl_3$ requires C, 34·5; H, 2·4; N, 8·9; Cl, 34·0%). It (3·8 g.) was hydrolysed by warming with concentrated sulphuric acid (20 c.c.) on the water-bath for 1 hour and pouring on ice; the *base* (VII) crystallised from aqueous alcohol in golden-orange needles, m. p. 121—122° (yield, 2·7 g.; 82·3%) (Found : C, 31·3; H, 2·1; N, 10·1; Cl, 38·8. $C_7H_5O_3N_2Cl_3$ requires C, 30·9; H, 1·8; N, 10·3; Cl, 39·2%). It was reconverted into its monoacetyl derivative by heating a toluene solution with an equimolecular proportion of acetyl chloride at 150° for 3 hours. The *diacetyl* derivative, obtained from the base (VII) (1 g.) or its monoacetyl derivative (1 g.) by heating with acetic anhydride (10 c.c.) at 150° for 3 hours in a sealed tube, crystallised from aqueous alcohol in colourless needles, m. p. 142—143° (yield, 80%) (Found : C, 37·5; H, 2·7; N, 8·3; Cl, 29·6. $C_{11}H_9O_5N_2Cl_3$ requires C, 37·1; H, 2·5; N, 7·9; Cl, 30·0%).

2 : 3 : 5-*Trichloro*-4-*nitro*-6-*methoxy*-N-*nitroaniline* (VI).—(a) Nitric acid (*d* 1·5; 30 c.c.) was added slowly to a stirred mixture of 3 : 4 : 6-trichloro-*o*-anisidine (5 g.) in acetic acid (20 c.c.) at <10° and the temperature kept at 10° for 1 hour. After addition to ice, the solid was filtered off, washed with water, dissolved in 20% aqueous sodium carbonate at 40°, and filtered. Acidification of the filtrate gave the N-*nitroamine* (VI), which crystallised from aqueous alcohol in colourless leaflets, m. p. 116—117° (decomp.) (yield, 4·8 g.; 69%) (Found : C, 26·8; H, 1·5; N, 13·0; Cl, 33·5. $C_7H_4O_5N_3Cl_3$ requires C, 26·6; H, 1·3; N, 13·3; Cl, 33·7%). (b) 3 : 4 : 5-Trichloro-5-nitro-*o*-anisidine (2 g.) in acetic acid (8 c.c.) and nitric acid (*d* 1·5; 20 c.c.) similarly gave the N-nitroamine (yield, 1·6 g.; 68·8%).

2 : 3 : 5-*Trichloro*-6-*methoxy*-p-*benzoquinone*.—Compound (VI) (4 g.) was refluxed with acetic acid (40 c.c.) for 2 hours, during which the almost colourless solution became deep red and oxides of nitrogen were evolved; after a further hour, nitrogen-free orange crystals separated from the cooled mixture; a further amount was obtained by concentrating the filtrate. The *quinone* crystallised from acetic acid in brilliant orange plates, m. p. 159° (yield, 1·8 g.; 59·4%) (Found : C, 34·4; H, 1·6; Cl, 43·9. $C_7H_3O_3Cl_3$ requires C, 34·8; H, 1·2; Cl, 44·1%).

2 : 3 : 5-*Trichloro*-6-*methoxybenzeneazo*-β-*naphthol*.—3 : 4 : 6-Trichloro-*o*-anisidine (6·8 g.) was heated with concentrated sulphuric acid (30 c.c.) and sodium nitrite (2·2 g.) on the water-bath until a drop of the solution gave no precipitate on dilution with water. The diluted diazo-solution was added to β-naphthol (4·4 g.) in 20% aqueous sodium hydroxide

(150 c.c.), or in acetic acid (250 c.c.), and the *azo*-dye was crystallised from toluene or acetic acid, forming orange-red needles, m. p. 166° (yield, 8·4 g., 73·5%, or 8·7 g., 76%, respectively) (Found : C, 52·9; H, 3·2; Cl, 27·6. $C_{17}H_{11}O_2N_2Cl_3$ requires C, 53·5; H, 2·9; Cl, 27·9%).

3 : 4 : 6-*Trichlorobenzene*-2-*diazo*-1-*oxide*.—Dry hydrogen chloride was passed into a solution of 3 : 4 : 6-trichloro-*o*-anisidine (4·5 g.) in dry chloroform (80 c.c.); the hydrochloride separated in colourless needles (yield, 5·1 g.; 96·8%). The latter was diazotised by grinding with concentrated hydrochloric acid and excess of sodium nitrite dissolved in the minimum amount of water; after 24 hours at 0°, the crystals were collected and recrystallised from ligroin, forming orange needles, m. p. 118° (decomp.), of the *diazo-oxide* (yield, 2·2 g.; 51·8%) (Found : N, 12·0; Cl, 47·1. $C_6HON_2Cl_3$ requires N, 12·5; Cl, 47·65%). The same compound was obtained when an aqueous solution of 3 : 4 : 6-trichloro-1-methoxybenzene-2-diazonium sulphate (from 4·5 g. of base) was heated on the water-bath (yield, 2 g.; 44·9%) or was neutralised with sodium acetate and kept overnight at 5—10° (yield, 2·2 g.; 49·3%). The diazo-oxide (1 g.) was heated with alcohol (20 c.c.) at 150° for 3 hours in a sealed tube, and the mixture added to water; 2 : 4 : 5-trichlorophenol separated in colourless needles, m. p. and mixed m. p. 63° (yield, 0·5 g.; 56·6%).

2 : 3 : 5-*Trichloro*-6-*hydroxybenzeneazo*-β-*naphthol*.—3 : 4 : 6-Trichlorobenzene-2-diazo-1-oxide (2·2 g.) in acetic acid (10 c.c.) was added to β-naphthol (1·4 g.) in sodium hydroxide (10 g.) and water (60 c.c.). Addition of acetic acid changed the deep red colour to blue and acidification precipitated the *azo*-dye, which crystallised from toluene in deep crimson needles, m. p. 226—228° (yield, 2·4 g.; 66%) (Found : C, 51·85; H, 2·45; N, 7·7; Cl, 28·2. $C_{16}H_9O_2N_2Cl_3$ requires C, 52·3; H, 2·45; N, 7·6; Cl, 29·0%).

3 : 4 : 6-*Trichloro*-5-*nitrobenzene*-2-*diazo*-1-*oxide* and 2 : 3 : 5-*Trichloro*-4-*nitro*-6-*hydroxybenzeneazo*-2′-*hydroxy*-3′-*naphthanilide* (VIII).—3 : 4 : 6-Trichloro-5-nitro-*o*-anisidine (2·7 g.) was diazotised with nitrosylsulphuric acid (prepared from 0·7 g. of sodium nitrite and 10 c.c. of sulphuric acid) on the water-bath, and the diazo-solution neutralised with sodium acetate. The diazo-oxide was collected after 6 hours (yield, 2 g.; 74·6%), dissolved in acetic acid (5 c.c.), and added to 2-hydroxy-3-naphthoic acid (2 g.) in aqueous sodium hydroxide; acidification with acetic acid gave the *azo*-dye, which crystallised from nitrobenzene in brownish-red plates with a bronze lustre, m. p. 285° (Found : C, 51·8; H, 2·5. $C_{22}H_{13}O_5N_4Cl_3$ requires C, 51·9; H, 2·4%).

2 : 3 : 5-*Trichloro*-4-*nitro*-6-*methoxybenzeneazo*-2′-*hydroxy*-3′-*naphthanilide*.—3 : 4 : 6-Trichloro-5-nitro-*o*-anisidine (2·7 g.) was diazotised and coupled with 2-hydroxy-3-naphthanilide (2·7 g.) in acetic acid (150 c.c.) at 40—50°. After several crystallisations from toluene, the *azo*-dye formed red needles, m. p. 282° (yield, 3·2 g.; 58·7%) (Found : C, 53·1; H, 2·7. $C_{24}H_{15}O_5N_4Cl_3$ requires C, 52·8; H, 2·75%); some of the hydroxyazo-dye (VIII) was also formed.

3 : 4 : 6-*Trichloro*-2 : 5-*diaminoanisole*.—Iron powder (4 g.) was added to 3 : 4 : 6-trichloro-5-nitro-*o*-anisidine (3·8 g.) in acetic acid (20 c.c.), alcohol (30 c.c.), and water (15 c.c.) at 70°; after refluxing for 1 hour, the mixture was filtered on ice, and the solid collected. The *diamine* crystallised from aqueous alcohol in colourless needles, m. p. 121—122°, turning pink in air (yield, 2·8 g.; 83%) (Found : C, 35·1; H, 2·9; N, 11·35; Cl, 43·7. $C_7H_7ON_2Cl_3$ requires C, 34·7; H, 2·8; N, 11·6; Cl, 44·1%). The 2 : 5-*diacetyl* derivative, obtained by refluxing the diamine (1 g.) with acetic anhydride (10 c.c.) for 4 hours, crystallised from aqueous alcohol in colourless needles, m. p. 342° (decomp.) (Found : C, 41·1; H, 3·5. $C_{11}H_{11}O_3N_2Cl_3$ requires C, 40·6; H, 3·4%). The 2-*monoacetyl* derivative (IX) was obtained by refluxing acetyl-3 : 4 : 6-trichloro-5-nitro-*o*-anisidine (6·5 g.) with iron powder (4 g.) in acetic acid (50 c.c.), alcohol (60 c.c.) and water (40 c.c.) for 1 hour; it separated from aqueous acetic acid in colourless cubes, m. p. 202° (yield 3·8 g.; 64·4%) (Found : C, 37·4; H, 3·1; N, 10·3; Cl, 37·4. $C_9H_9O_3N_2Cl_3$ requires C, 38·1; H, 3·2; N, 9·9; Cl, 37·6%). Compound (IX), dissolved in 50% acetic acid, was diazotised by pouring into water, hydrochloric acid and sodium nitrite, and coupled with alkaline β-naphthol; 2 : 3 : 6-*trichloro*-5-*methoxy*-4-*acetamidobenzeneazo*-β-*naphthol* crystallised from toluene in orange needles, m. p. 267—268° (Found : C, 52·1; H, 3·3. $C_{19}H_{14}O_3N_3Cl_3$ requires C, 52·0; H, 3·2%). 2-*Diacetyl*-3 : 4 : 6-*trichloro*-5-*amino*-*o*-*anisidine*, obtained by reducing the corresponding 5-nitro-compound (2 g.) under similar conditions to those used in making (IX), crystallised from aqueous acetic acid in colourless prismatic needles, m. p. 142° (yield, 1·2 g.; 69·6%) (Found : C, 40·3; H, 3·5; N, 8·5; Cl, 33·0. $C_{11}H_{11}O_3N_2Cl_3$ requires C, 40·6; H, 3·4; N, 8·6; Cl, 32·7%).

3 : 4 : 6-*Trichloro*-5-*bromo*-*o*-*anisidine*.—Compound (V) (4·6 g.) was dissolved in acetic acid (10 c.c.) at 15°, and excess of bromine in acetic acid added gradually until crystals separated progressively; these were filtered off, warmed with concentrated sulphuric acid on the water-bath, and poured on ice. 3 : 4 : 6-*Trichloro*-5-*bromo*-*o*-*anisidine* crystallised from alcohol in colourless needles, m. p. 101° (yield, 6·1 g.; 98·4%) (Found : C, 27·9; H, 1·9; 6·412 mg. gave 13·600 mg. of AgCl + AgBr. $C_7H_5ONCl_3Br$ requires C, 27·5; H, 1·6%; 13·140 mg. of AgCl + AgBr). The *monoacetyl* derivative crystallised from aqueous alcohol in colourless, prismatic needles, m. p. 236—237° (Found : C, 32·1; H, 2·4; 0·221 mg. gave 0·384 mg. of AgCl + AgBr. $C_9H_7O_2NCl_3Br$ requires C, 31·4; H, 2·0%; 0·394 mg. of AgCl + AgBr). 2 : 3 : 5-*Trichloro*-4-*bromo*-6-*methoxybenzeneazo*-β-*naphthol*, prepared by diazotising the base (6·1 g.) with nitrosylsulphuric acid (prepared from 1·4 g. of sodium nitrite and 20 c.c. of sulphuric acid) on the water-bath and coupling with alkaline β-naphthol, crystallised from toluene in bright red needles, m. p. 195° (yield, 6·8 g.; 73·9%) (Found : 0·204 mg. gave 0·275 mg. of AgCl + AgBr. $C_{17}H_{10}O_2N_2Cl_3Br$ requires 0·276 mg. of AgCl + AgBr).

When 3 : 4 : 6-trichloro-5-bromo-*o*-anisidine (2·1 g.) was diazotised with nitrosylsulphuric acid, diluted, sodium acetate added, and the solution kept at room temperature for 1 day, the 2-diazo-1-oxide separated (yield, 1·8 g.; 85·7%); it coupled with 2-hydroxy-3-naphthanilide in aqueous sodium hydroxide, and acidification with acetic acid gave 2 : 3 : 5-*trichloro*-4-*bromo*-6-*hydroxybenzeneazo*-2′-*hydroxy*-3′-*naphthanilide*, which crystallised from acetic acid in reddish-brown plates with a bronze lustre, m. p. 274° (Found : N, 7·4; 3·842 mg. gave 3·810 mg. of AgCl + AgBr. $C_{23}H_{12}O_3N_2Cl_3Br$ requires N, 7·4%; 4·202 mg. of AgCl + AgBr).

3 : 4 : 5 : 6-*Tetrachloro*-*o*-*anisidine* (cf. Bureš and Havlinova, *loc. cit.*).—Dry chlorine was introduced into 3 : 4 : 6-trichloro-*o*-anisidine (4·5 g.) in chloroform (80 c.c.). The colourless solid which soon separated was warmed with concentrated sulphuric acid on the water-bath until evolution of hydrogen chloride ceased, and the mixture poured on ice. Tetrachloro-*o*-anisidine crystallised from aqueous alcohol in colourless needles, m. p. 95° (yield, 4 g.; 76·9%) (Found : N, 5·3; Cl, 54·5. Calc. for $C_7H_5ONCl_4$ : N, 5·4; Cl, 54·4%). The base (1·3 g.) was diazotised with nitrosylsulphuric acid (prepared from 0·35 g. of sodium nitrite and 6 c.c. of sulphuric acid) on the water-bath for 15 minutes and coupled with alkaline β-naphthol; 2 : 3 : 4 : 5-*tetrachloro*-6-*methoxybenzeneazo*-β-*naphthol* crystallised from toluene in bright red needles, m. p. 204° (yield, 1·5 g.; 71·4%) (Found : N, 6·55; Cl, 33·9. $C_{17}H_{10}O_2N_2Cl_4$ requires N, 6·7; Cl, 34·1%).

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## 60. A Synthesis of Methacrylic Acid.

By Theodore White.

The synthesis of methacrylic acid has been achieved by oxidation of methyl *iso*propenyl ketone with sodium hypochlorite. The preparation of two new esters is described.

The importance in plastics chemistry of polymeric materials formed from derivatives of methacrylic acid has led to the development of a number of syntheses of this αβ-unsaturated acid and its esters, some of which are described only in the patent literature. Frankland and Duppa (*Annalen*, 1865, **136**, 12) first prepared the esters by treating α-hydroxy*iso*butyric esters with phosphorus trichloride, and the related dehydrohalogenation of α- or β-halogenated *iso*butyric esters was used by Walden (*Z. physikal. Chem.*, 1896, **20**, 574) and has since been the subject of several patents, *e.g.*, Bauer (B.P., 1930, 316,547) and Barrett (U.S.P., 1935, 2,013,048). Ritchie, Jones, and Burns (J., 1935, 714) prepared methyl methacrylate by pyrolytic removal of acetic acid from methyl α-acetoxy*iso*butyrate, and an alternative method of preparation from acetone cyanohydrin by treatment with hot concentrated sulphuric acid and with methyl alcohol devised by Crawford (B.P., 1932, 405,699) and by Hill (B.P., 1938, 490,007) is now in extensive use. The latter method constitutes by far the most convenient way of obtaining the acid itself.

A further method, based on Einhorn's observation (*Annalen*, 1888, **243**, 362) that aromatic αβ-unsaturated ketones of the type R·CH:CH·CO·$CH_3$ can be oxidised by hypohalogenites to the corresponding unsaturated acid R·CH:CH·$CO_2$H, has now been devised. In the course of work on polymerisable αβ-unsaturated aliphatic ketones (cf. White and Haward, this vol., p. 25) it was observed that the combination of electronic effects present in these compounds rendered the carbon–carbon double bond remarkably inert to electrophilic reagents and caused it to react preferably with such nucleophilic reagents as would normally add on only to a carbonyl group. This enabled such unsaturated ketones to be satisfactorily oxidised by hypohalogenites similarly to the corresponding aromatic ketones or the saturated aliphatic ketones, the reaction proceeding thus :

$$CH_2{:}CR{\cdot}CO{\cdot}CH_3 + 3NaOCl = CH_2{:}CR{\cdot}CO_2Na + 2NaOH + CHCl_3.$$

Methyl vinyl ketone and methyl *iso*propenyl ketone were successfully oxidised in this manner to acrylic acid and methacrylic acid respectively, despite the tendency displayed by both ketones to resinification in the presence of aqueous alkali solutions and their tendency to add on hypochlorous acid to give the corresponding α-chloro-β-hydroxy-ketones. The oxidation of methyl vinyl ketone proceeded smoothly with bleaching powder or hypohalogenites in general, but the difficulty of extracting the resultant acrylic acid (owing to its great solubility in water and tendency to polymerise) made the preparation one of mainly theoretical interest.

The oxidation of methyl *iso*propyl ketone to methacrylic acid proved to be rather more difficult owing to the fact that the inductive effect of the α-methyl group tended to favour the addition of hypochlorous acid to the αβ-double bond, this tendency being so marked that in attempted oxidations with bleaching powder and solutions of calcium hypochlorite only the hypochlorous acid addition product was formed. However, by using strongly alkaline solutions of sodium hypochlorite, and by paying particular care to the conditions of the reaction, it was found that both resinification and the addition of hypochlorous acid were avoided and that the reaction proceeded smoothly to give equivalent amounts of sodium methacrylate and chloroform. The suppression of the addition of hypochlorous acid in these circumstances would appear to be related to the greater stability of the hypochlorite ion in the presence of sodium hydroxide than in the presence of calcium ions, where the tendency to formation of hypochlorous acid predominates. On neutralising such strongly alkaline solutions of sodium hypochlorite with sulphuric acid, saturated sodium sulphate solutions were produced, from which the methacrylic acid was salted out in a state of fairly high purity, and the pure acid was readily obtained by drying and redistillation of this product.

Some esters of methacrylic acid are preferably prepared, not by esterification of the acid, but by alcoholysis from monomeric methyl methacrylate along the lines laid down by Izard (B.P., 1937, 470,503). *n-Hexyl methacrylate* and *ethylene dimethacrylate* were thus prepared, and since the marked tendency of these products to polymerise gives rise to practical difficulties which are absent from similar reactions with non-polymerisable esters, the details of the methods are recorded.

### Experimental.

*Methyl* iso*Propenyl Ketone.*—A mixture of methyl ethyl ketone (4 l.) and paraformaldehyde (600 g.) was stirred with 2N-methyl-alcoholic potash (20 ml.) (*ca.* 3 hours) until a test portion no longer reduced boiling ammoniacal silver nitrate solution. The mixture was then brought to pH 6·9 with 2N-acetic acid in methyl ethyl ketone, and the excess of ketone distilled off up to 100°. The remaining product was fractionally distilled under reduced pressure with a Fenske column of the type used by White and Haward (*loc. cit.*), giving ketomethylbutanol (1 kg.), b. p. 84°/19 mm. This distillate was treated with concentrated sulphuric acid (20 g.), and gave, on heating to distillation point, a water–methyl *iso*propenyl ketone mixture which distilled at 94—96°. The aqueous layer was separated. The methyl *iso*propenyl ketone was dried by shaking twice with anhydrous sodium sulphate and keeping overnight over anhydrous copper sulphate; on redistillation through a Fenske column, it gave pure methyl *iso*propenyl ketone (700 g.), b. p. 97·5—98°/760 mm.

*Methacrylic Acid by Oxidation of Methyl* iso*Propenyl Ketone.*—Since concentrated sodium hypochlorite solution tends to decompose during its preparation from chlorine and concentrated sodium hydroxide solution, the following method was normally adopted : " Maxochlor " (I.C.I. high chlorine content soluble dry bleach) (400 g.) was gradually added,

with stirring and external cooling, to sodium hydroxide (360 g.) in water (1 l.), the temperature being maintained below 15°. The mixture was then stirred for 30 minutes and filtered, giving 3N-sodium hypochlorite in 6N-sodium hydroxide.

This solution was stirred in a flask cooled in water, and methyl *iso*propenyl ketone (84 g.) added at such a rate that the temperature remained below 20°. The mixture was stirred and cooled for a further hour, and the chloroform (50 g.) then allowed to settle and separated. The aqueous solution was acidified with 50% sulphuric acid, the temperature being maintained below 30°, and an upper layer of methacrylic acid salted out. This was separated, dried with anhydrous sodium sulphate, and redistilled under reduced pressure in the presence of 1% of quinol in an atmosphere of oxygen-free nitrogen, giving a colourless pungent acid, b. p. 63°/10 mm., $n_D^{20°}$ 1·429 (Found: equiv., 86). Yield, 32 g.; *i.e.*, 41% (methacrylic acid has b. p. 65°/12 mm., $n_D^{20°}$ 1·431, equiv., 86). The product added on the theoretical amount of bromine and polymerised readily to a hard white mass with peroxides or in the presence of light.

*Ethylene Dimethacrylate.*—A mixture of monomeric methyl methacrylate (750 g.), ethylene glycol (100 g.), concentrated sulphuric acid (5 g.), and quinol (50 g.) was heated at 150° for 5 hours in a current of oxygen-free nitrogen. The distillate was fractionated through a Fenske column with the reflux ratio so adjusted as to maintain the temperature of distillation at 64—65° (that of the methyl alcohol–methyl methacrylate binary mixture). After 5 hours the temperature rose steadily and the heating was then discontinued, 170 ml. of distillate having been collected containing 125 ml. of methyl alcohol (corresponding to 100% alcoholysis). The reaction mixture was then cooled, neutralised with solid sodium bicarbonate, washed with water, dried over calcium chloride overnight, and fractionated through a Fenske column under reduced pressure; the fraction, b. p. 80—140°/15 mm., was refractionated to give a small amount of product boiling up to 120°/15 mm. and a major portion, b. p. 122—126°/15 mm., $n_D^{20°}$ 1·4558 (Found: C, 60·4; H, 7·3; equiv., by saponification. 200. $C_{10}H_{14}O_4$ requires C, 60·6; H, 7·1%; equiv., 198). Yield, 90 g.; *i.e.*, 46%. This product was *ethylene dimethacrylate*, which, in the presence of benzoyl peroxide, polymerised to a hard, colourless, insoluble, cross-linked resin.

n-*Hexyl Methacrylate.*—A mixture of monomeric methyl methacrylate (2850 g.), *n*-hexyl alcohol (656 g.), precipitated sulphur (20 g.), and concentrated sulphuric acid (20 ml.) was refluxed in an atmosphere of oxygen-free nitrogen until the temperature at the head of a Fenske column was 64—65°, the distillate being then taken off as rapidly as possible without rise of the distillation temperature. 285 Ml. of distillate (containing 220 ml. of methyl alcohol) were obtained at 65°, 65 ml. (containing 20 ml. of methyl alcohol) at 65—100°, and 1500 ml. of methyl methacrylate at 100°. The residual mixture was treated with 36 g. of powdered calcium carbonate to neutralise the acid present. A further 400 ml. of methyl methacrylate were then distilled off at 100° and the remaining product was fractionated through a Fenske column under reduced pressure to give methyl methacrylate (170 ml.), b. p. 20°/17 mm., *n*-hexyl alcohol (125 g.), b. p. 67°/17 mm., and n-*hexyl methacrylate*, b. p. 86—88°/17 mm., $n_D^{20°}$ 1·4300 (Found: C, 70·3; H, 10·8; equiv., by saponification, 169. $C_{10}H_{18}O_2$ requires C, 70·6; H, 10·6%; equiv., 170). The yield was 735 g., *i.e.*, 83%, calculated on the *n*-hexyl alcohol. The product polymerised readily to a highly extensible, tacky polymer.

The author is grateful to Messrs. Colmore Adhesives, Ltd., for permission to publish the above work.

COLMORE ADHESIVES, LTD., TEAM VALLEY TRADING ESTATE,
GATESHEAD-ON-TYNE.

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## 61. *Polymethylbenzoylnaphthoic Acids.*

By RICHARD H. MARTIN.

Naphthalene-1 : 2-dicarboxylic anhydride has been condensed by the Friedel–Crafts reaction with 1 : 2 : 3 : 4-tetramethylbenzene and with hemimellithene. The latter has also been condensed with 2-methyl-1-naphthoyl chloride. 1-(3′ : 4′ : 5′-*Trimethylbenzoyl*)-2-*methylnaphthalene* has been oxidised by selenium dioxide to 1-(3′ : 4′ : 5′-*trimethylbenzoyl*)-2-*naphthoic acid*.

MANY polymethyl-1 : 2-benzanthracenes have powerful carcinogenic activity (see Badger *et al.*, *Proc. Roy. Soc.*, 1940, *B*, **129**, 439). The most favourable positions of substitution in the 1 : 2-benzanthracene molecule are 5, 9, 10, and, to a less extent, 6. Simultaneous substitution at two or three of these positions gives rise to the most potent carcinogenic hydrocarbons known (*e.g.*, 9 : 10-dimethyl-1 : 2-benzanthracene). It was, therefore, considered of interest to investigate the effect of the simultaneous introduction of methyl groups into "favourable" and "less favourable" positions of the 1 : 2-benzanthracene molecule (*e.g.*, 5 : 6 : 7-, 6 : 7 : 8-, 5 : 6 : 7 : 8-). The work here described is preliminary and it is recorded because circumstances have prevented its completion (see also Bachmann and Chemerda, *J. Org. Chem.*, 1941, **6**, 36).

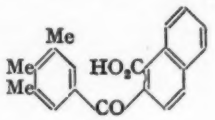
The results may be summarised as follows: 1. The Friedel–Crafts reaction between hemimellithene and naphthalene-1 : 2-dicarboxylic anhydride (in an excess of hemimellithene) gives a mixture of 2-(3′ : 4′ : 5′-*trimethylbenzoyl*)-1-*naphthoic acid* (I) and 1-(3′ : 4′ : 5′-*trimethylbenzoyl*)-2-*naphthoic acid* (II), the former preponderating. The above constitutions of the two acids were assigned on the basis of the results of potash fusions. This potash fusion method is not conclusive (cf. Huntress *et al.*, *J. Amer. Chem. Soc.*, 1942, **64**, 2845), but the oxidation of 1-(3′ : 4′ : 5′-*trimethylbenzoyl*)-2-*methylnaphthalene* with selenium dioxide (see below) affords complete proof of the correctness of the constitutions given.

2. The condensation between the magnesium derivative of 4-bromohemimellithene (preparation improved) and naphthalene-1 : 2-dicarboxylic anhydride gives a mixture of acids difficult to separate.

3. The Friedel–Crafts reaction between hemimellithene and 2-methyl-1-naphthoyl chloride (in carbon disulphide) gives a mixture of 1-(2′ : 3′ : 4′-*trimethylbenzoyl*)-2-*methylnaphthalene* and 1-(3′ : 4′ : 5′-trimethylbenzoyl)-2-methylnaphthalene. The latter on oxidation with selenium dioxide in a sealed tube furnishes 1-(3′ : 4′ : 5′-trimethylbenzoyl)-2-naphthoic acid (II), which was identified as its *acetoxy-lactone*. It is identical with the acetoxy-lactone of the acid obtained as described under (1). This acid was accompanied by other acids which were not further examined.

Preliminary attempts to cyclise 2-(3′ : 4′ : 5′-trimethylbenzoyl)-1-naphthoic acid (I) with benzoyl chloride and a few drops of concentrated sulphuric acid (Waldmann, *J. pr. Chem.*, 1938, **150**, 121) were unsuccessful.

4. The Friedel–Crafts reaction between 1 : 2 : 3 : 4-tetramethylbenzene (Smith and Cass, *J. Amer. Chem. Soc.*, 1932, **54**, 1614) and naphthalene-1 : 2-dicarboxylic anhydride (in nitrobenzene) gives 2-(2′ : 3′ : 4′ : 5′-*tetramethylbenzoyl*)-1-*naphthoic acid* (III) or less probably 1-(2′ : 3′ : 4′ : 5′-*tetramethylbenzoyl*)-2-*naphthoic acid*. The choice between the two formulæ was made on account of the colour reaction with concentrated sulphuric acid (cf. Cook, J., 1932, 454, and the above cases). A yellow crystalline compound, the analysis of which agrees with the formula of 5 : 6 : 7 : 8-*tetramethyl*-1 : 2-*benzanthraquinone*, is produced when the above substituted naphthoic acid is treated with benzoyl chloride and a few drops of concentrated sulphuric acid.



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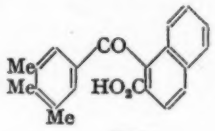

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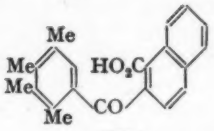

(III.)

## Experimental.

2-(3′ : 4′ : 5′-*Trimethylbenzoyl*)-1-*naphthoic Acid* (I) *and* 1-(3′ : 4′ : 5′-*trimethylbenzoyl*)-2-*naphthoic Acid* (II).—Powdered aluminium chloride (18 g.) is added in small portions to an ice-cold, finely divided suspension of naphthalene-1 : 2-dicarboxylic anhydride (12 g.) in hemimellithene (40 c.c.). The mixture is left for 6 hours at room temperature and then decomposed with ice and hydrochloric acid. The solid is extracted with 2N-sodium hydroxide and filtered from some neutral material (6·9 g.). This neutral fraction is unaffected by refluxing for 1 hour with 20% alcoholic potassium hydroxide. It has not been further investigated. The acids are precipitated from the alkaline solution with hydrochloric acid.

(*a*) 1-(3′ : 4′ : 5′-*Trimethylbenzoyl*)-2-*naphthoic acid* (II) is isolated through its insoluble ammonium salt and purified by crystallisation of its sodium salt. The free acid is then recrystallised successively from acetic acid and benzene–ethyl alcohol. It forms a colourless microcrystalline powder (1·6 g.), m. p. 273—274°, which gives a green colour with concentrated sulphuric acid (Found: C, 78·7; H, 5·7. $C_{21}H_{18}O_3$ requires C, 78·9; H, 5·7%).

*Acetoxy-lactone.* A solution of the acid (1 g.) in anhydrous pyridine (5 c.c.) and acetic anhydride (1·5 c.c.) is heated on the water-bath for 2 hours. The solid separating after dilution with water is recrystallised from benzene, separating as a white microcrystalline powder, m. p. 231—232° (Found: C, 76·8; H, 5·7. $C_{23}H_{20}O_4$ requires C, 76·6; H, 5·6%). By hydrolysis (alcoholic potash) the acid, m. p. 273—274°, is recovered.

(*b*) 2-(3′ : 4′ : 5′-*Trimethylbenzoyl*)-1-*naphthoic acid* (I), obtained by acidification of the ammonia solution after separation of the insoluble salt of (II), is purified through its sparingly soluble sodium salt. The free acid is recrystallised from acetic acid and then xylene. It forms small white needles (5·4 g.), m. p. 191—192°. This acid gives a reddish-brown colour with concentrated sulphuric acid (Found: C, 78·9; H, 5·4. $C_{21}H_{18}O_3$ requires C, 78·9; H, 5·7%).

The *acetoxy-lactone* (prepared as above) separates from ethyl alcohol as a white microcrystalline powder, m. p. 161·5—162·5° (Found: C, 76·6; H, 5·6. $C_{23}H_{20}O_4$ requires C, 76·6; H, 5·6%). By hydrolysis it regenerates the acid, m. p. 191—192°.

The *benzoyloxy-lactone* is prepared by means of benzoyl chloride in pyridine as for the acetoxy-lactone. The oily product is obtained crystalline by treatment with methyl alcohol and recrystallised from benzene–ethyl alcohol. It forms white microscopic needles, m. p. 191·5—192·5° (Found: C, 78·7; H, 5·4. $C_{28}H_{22}O_4$ requires C, 78·9; H, 5·3%).

*Degradation of Acids* (I) *and* (II) (cf. Cook, *loc. cit.*).—(*a*) The acid (II) (0·5 g.) is added in small portions to potassium hydroxide (4 g.) at 280°. After ½ hour at 340° the mass is cooled and extracted with hot water (charcoal) and the acids are precipitated with hydrochloric acid. After three recrystallisations from ethyl alcohol, 3 : 4 : 5-trimethylbenzoic acid separates in transparent prisms, m. p. 215·5—216° (Jacobson, *Ber.*, 1882, **15**, 1853). From the mother-liquor, 2-naphthoic acid is isolated with difficulty, m. p. 172—174° (not depressed by an authentic specimen, m. p. 182°).

(*b*) The acid (I) is added to potassium hydroxide as above, but the whole reaction is carried out between 260—280°; 3 : 4 : 5-trimethylbenzoic acid, m. p. 215·5—216·5°, and 1-naphthoic acid, m. p. 154°, are isolated in the same way as under (*a*).

4-*Bromo*-1 : 2 : 3-*trimethylbenzene*. This compound was prepared according to Smith and Moyle (*J. Amer. Chem. Soc.*, 1936, **58**, 1) but the reaction mixture was kept for 18 hours at 0° and then 2 hours at room temperature. Yield, 89%; b. p. 128°/25 mm. (Smith and Moyle, *loc. cit.*, claim 47% yield, b. p. 103—103·5°/12 mm.).

1-(2′ : 3′ : 4′-*Trimethylbenzoyl*)- *and* 1-(3′ : 4′ : 5′-*Trimethylbenzoyl*)-2-*methylnaphthalene*.—Powdered aluminium chloride (4 g.) is added in small portions to a solution of 2-methyl-1-naphthoyl chloride (prepared from 5 g. of the acid by refluxing with thionyl chloride, followed by distillation under reduced pressure) in carbon disulphide (35 c.c.) containing 4 c.c. of hemimellithene and cooled in ice. The mixture is kept for 40 hours at 0° and 24 hours at room temperature (with frequent shaking) and then decomposed with ice and hydrochloric acid and volatile products are steam-distilled. The non-volatile residue is extracted with ether and washed with aqueous sodium carbonate. The two ketones formed are separated by crystallisation from acetic acid.

The less soluble 1-(3′ : 4′ : 5′-*trimethylbenzoyl*)-2-*methylnaphthalene* is purified by sublimation at 170°/0·1 mm. and recrystallised from acetic acid and then from methyl alcohol. It forms microscopic transparent prisms (2·8 g.), m. p. 150—151° (Found: C, 87·2; H, 7·1. $C_{21}H_{20}O$ requires C, 87·4; H, 7·0%).

1-(2′ : 3′ : 4′-*Trimethylbenzoyl*)-2-*methylnaphthalene*, isolated from the acetic acid mother-liquor, is sublimed at 90°/0·2 mm. and then crystallised successively from acetic acid and methyl alcohol. It forms microscopic transparent prisms, m. p. 108—108·5° (Found: C, 87·1; H, 6·8%).

*Oxidation of* 1-(3′ : 4′ : 5′-*Trimethylbenzoyl*)-2-*methylnaphthalene with Selenium Dioxide* (cf. Cook, J., 1932, 1476).—The ketone (1·5 g.) is heated at 235° for 4 hours in a sealed tube with selenium dioxide (3 g.) and water (3 c.c.). The products are collected, washed with water, and extracted with aqueous sodium carbonate. The precipitated acids (1·1 g.) are heated on the water-bath for 1 hour with acetic anhydride in pyridine. The benzene solution of the reaction products is washed with aqueous sodium carbonate. By addition of hydrochloric acid to this solution an appreciable amount of acidic products is precipitated (formed possibly by oxidation of one or more of the methyl groups in the benzene ring). The neutral fraction gives after purification the acetoxy-lactone of 1-(3′ : 4′ : 5′-trimethylbenzoyl)-2-naphthoic acid (II), m. p. 231—232°, not depressed by the specimen obtained above. Attempts to reduce the amount of acids formed as by-product by altering the conditions of reaction were unsuccessful; *e.g.*, 3 hours at 235° gave the same result and 2 hours at 215° gave only a very small amount of total acids.

2-(2′ : 3′ : 4′ : 5′-*Tetramethylbenzoyl*)-1-*naphthoic Acid* (III).—Aluminium chloride (35 g.) is added in small portions

to a well-stirred suspension of finely divided naphthalene-1 : 2-dicarboxylic anhydride (25 g.) in nitrobenzene (150 c.c.), the temperature being maintained at 10—15°. The mixture is then cooled to 0°, and 1 : 2 : 3 : 4-tetramethylbenzene (16·8 g.) added drop by drop. The reaction mixture is stirred for 12 hours at 0° and then 60 hours at room temperature. The complex is decomposed with ice and hydrochloric acid, the nitrobenzene steam-distilled, the residual aqueous solution decanted, and the steam-distillation carried on in the presence of 2N-sodium carbonate. The carbonate solution is heated with charcoal, filtered, and the acid is precipitated with hydrochloric acid. The acid is purified through its sparingly soluble sodium salt, followed by crystallisation from acetic acid. It forms a white powder, m. p. 241·5—242·5° (Found: C, 79·2; H, 5·8. $C_{22}H_{20}O_3$ requires C, 79·5; H, 6·1%). It gives a reddish-brown colour with concentrated sulphuric acid. Yield, 13 g. (m. p. 240°). Numerous alterations of the above procedure failed to improve the yield of this acid.

5 : 6 : 7 : 8-*Tetramethyl*-1 : 2-*benzanthraquinone* (?).—The acid (III) (1 g.) is heated on the water-bath with benzoyl chloride (2 c.c.) and concentrated sulphuric acid (2 drops) for 1 hour. The cold solution is poured into water, boiled, and the solid collected. This solid, after being boiled with 2N-sodium carbonate and then water, is sublimed at 200°/0·2 mm. and recrystallised from xylene. It separates as a microcrystalline yellow powder, m. p. 203—203·5° (Found: C, 83·9; H, 5·95. $C_{22}H_{18}O_2$ requires C, 84·1; H, 5·75%).

The author thanks Professor J. W. Cook, F.R.S., for his interest in the investigation and the British Empire Cancer Campaign for a grant to this Institute, which supported the work.

THE CHESTER BEATTY RESEARCH INSTITUTE, THE ROYAL CANCER HOSPITAL (FREE), LONDON, S.W.3. [*Received, March 31st*, 1943.]

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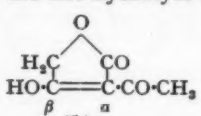
## 62. *Synthesis of Some α-Acyltetronic Acids.*

By WILSON BAKER, K. D. GRICE, and A. B. A. JANSEN.

α-Acetyltetronic acid (I) is readily synthesised from β-anilinocrotonic ester and chloroacetyl chloride. The methyl group of (I) is reactive and condenses with benzaldehyde, anisaldehyde, cinnamaldehyde and furfuraldehyde to give unsaturated compounds of the type (II), which have been reduced to the saturated α-acyltetronic acids (III). The furfurylidene derivative is reduced simultaneously to the tetrahydrofuran compound. The oxime of α-acetyltetronic acid is convertible into *α-acetamidotetronic acid*.

DERIVATIVES of α-acyltetronic acids are frequently found as products of mould metabolism, and the synthesis of such compounds is, therefore, of some interest. The only successful method previously described is due to Benary (*Ber.*, 1909, **42**, 3912), who condensed chloroacetyl chloride with ethyl β-aminocrotonate in pyridine, cyclised the resulting α-chloroacetyl-β-aminocrotonic ester with loss of ethyl chloride by heating to give "α-acetyltetronamide," and deaminated this by alkaline hydrolysis, thus obtaining α-acetyltetronic acid (I). The yield was not stated. Benary also describes the preparation by similar methods of α-acetyltetronanilide, but did not hydrolyse it to (I).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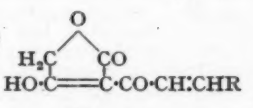
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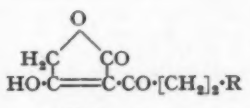
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The preparation of α-acetyltetronanilide has been considerably simplified, and it is now shown that it is readily hydrolysed in cold alkaline solution to α-acetyltetronic acid (I), which may be directly extracted from the acidified solution. The overall yield of (I) from molecular quantities of methyl or ethyl acetoacetate and chloroacetyl chloride is 34%.

The methyl group of α-acetyltetronic acid is, as expected, less reactive than that in a simple methyl ketone. All tetronic acids are very powerful acids, the anion being stabilised by a resonance distribution of the charge between the oxygen atoms of the acidic hydroxyl group and of the ester carbonyl group (see Kumler, *J. Amer. Chem. Soc.*, 1938, **60**, 859) (the α-carbon atom is also involved to a certain extent, since its chemical behaviour resembles that of an *o*-carbon atom in a phenol). In α-acetyltetronic acid the charge is also distributed on the oxygen atom of the α-carbonyl group, thus making it an even stronger acid, but at the same time the methyl group shows diminished reactivity owing to its partial ionic character, $:C(O^-)\cdot CH_2$. In confirmation it has been found that both α-acetyl- and α-carbethoxy-tetronic acids are appreciably stronger acids than tetronic acid, the p$K$ values being for tetronic acid, 3·76 (Kumler, *loc. cit.*), α-acetyl- and α-carbethoxy-tetronic acids approximately 1·8. We wish to express our thanks to Dr. E. P. Abraham of the Sir William Dunn School of Pathology, Oxford, for the determination of the last two values.

Aldehydes do not condense with the methyl group of (I) in alkaline solution but react in glacial acetic acid solution in presence of a little piperidine to give poor yields of condensation products which are not easily isolated. In this way (I) has been condensed with benzaldehyde, cinnamaldehyde, furfuraldehyde and anisaldehyde to give the unsaturated derivatives (II; R = Ph, Ph·CH:CH, $C_4H_3O$, and *p*-MeO·$C_6H_4$), α-(β-*phenylacrylyl*)-, α-(β-*styrylacrylyl*)-, α-(β-*furylacrylyl*)-, and α-(p-*methoxyphenylacrylyl*)-*tetronic acids* respectively. Catalytic reduction of the first three of these substances yielded the saturated α-acyltetronic acids (III; R = Ph, Ph·$CH_2$·$CH_2$, and $C_4H_7O$), α-(β-*phenylpropionyl*)-, α-(δ-*phenylvaleryl*)-, and α-(β-2-*tetrahydrofurylpropionyl*)-*tetronic acids*; in the case of the furfurylidene derivative the furan nucleus was also reduced.

The oxime of α-acetyltetronic acid undergoes the Beckmann transformation when treated with phosphorus pentachloride in phosphorus trichloride, and is converted into *α-acetamidotetronic acid*.

## Experimental.

*α-Acetyltetronic Acid* (I).—(1) *Methyl β-anilinocrotonate.* A mixture of methyl acetoacetate (150 g. or ethyl acetoacetate, 168 g.), aniline (120 g.), and a drop of concentrated hydrochloric acid as catalyst (see Coffey, Thompson, and Wilson, J., 1936, 856) was kept overnight, the separated water distilled off under diminished pressure on the water-bath, and the last traces of water removed by similar distillation after the addition of benzene (100 c.c.). The oily residue solidified completely on seeding in the case of the methyl ester. (2) *Anilide of α-acetyltetronic acid.* To the preceding product, dissolved in dry ether (400 c.c.) and anhydrous pyridine (102 g.; 1 mol.), was added during 1 hour chloroacetyl chloride (145 g.; 1 mol.) in dry ether (150 c.c.) whilst cooling in ice and shaking; after 1½ hours the ether was removed on the water-bath. Methyl or ethyl chloride was slowly evolved from the residue when heated on the water-bath for 1½ hours and then in an oil-bath for 4 hours while the temperature was slowly raised from 120° to 130°. To the light brown solid, water (1 l.) was added; after heating, the mass was powdered, collected, washed well with water, and dried on the steam-bath (yield, 164 g.). This crude anilide was refluxed for 1 hour with alcohol (500 c.c.), cooled, collected, washed twice with alcohol (200 c.c. each time) and dried (yield, 121 g.; m. p. 180—184°; lit. m. p. 188—189°). (3) *α-Acetyltetronic acid.* A mixture of the anilide (121 g.) and a solution of sodium hydroxide (30 g.) in water (450 c.c.) was shaken for 24 hours, filtered after shaking with ether (150 c.c.), and the aqueous layer again extracted with ether (75 c.c.). After acidification with concentrated hydrochloric acid (120 c.c.) and addition of salt (150 g.) the solution was extracted 16 times with chloroform (75 c.c. each time); the extracts after drying over sodium sulphate were distilled (the last few c.c. of chloroform were removed in a current of air while warm), leaving α-acetyltetronic acid (67 g.; m. p. 78—80°). By crystallisation in portions from light petroleum (b. p. 80—100°) the pure, colourless product was obtained (yield, 63 g.; m. p. 81—82°; lit. m. p. 79·5—80·5°).

*α-(β-Phenylacrylyl)tetronic Acid* (II; R = Ph).—α-Acetyltetronic acid (7·1 g.), benzaldehyde (5·3 g.; 1 mol.), glacial acetic acid (25 c.c.), and piperidine (1 c.c.) were heated on the water-bath for 2½ hours, and stirred into water (200 c.c.). The dark red product was collected, dried, dissolved in chloroform (150 c.c.), and shaken in portions with 0·5% aqueous sodium hydroxide (500 c.c. in all). The aqueous layer was filtered and acidified at 40°, giving a yellow, amorphous product (2·4 g.), which was crystallised first from methyl alcohol (65 c.c.; charcoal), and then from light petroleum (650 c.c.; b. p. 80—100°), being obtained in yellow or light brown needles (1 g.), m. p. 138—140° (Found in material again crystallised from light petroleum: C, 67·8; H, 4·4. $C_{13}H_{10}O_4$ requires C, 67·8; H, 4·4%). In dilute alcoholic solution it gives an orange ferric chloride reaction, and its solutions in alkali are colourless.

*α-(β-Phenylpropionyl)tetronic Acid* (III; R = Ph).—The preceding acid (0·75 g.) in N-sodium hydroxide (4 c.c.) and ethyl alcohol (10 c.c.) was shaken in hydrogen in presence of Raney nickel. Reduction, which was at first rapid, almost ceased after the uptake of hydrogen corresponding to 1 molecule. The liquid was filtered and acidified; the solid thus obtained crystallised from light petroleum (b. p. 80—100°) in colourless, flat prisms (0·54 g.), m. p. 131° after softening at 128° (Found: C, 67·0; H, 5·2. $C_{13}H_{12}O_4$ requires C, 67·2; H, 5·2%). It is slightly soluble in water and the solution gives an orange precipitate with ferric chloride. Hydrolysis with 2N-sulphuric acid for 4 hours gave, after removal of acetol by distillation of the neutralised solution, a 50% yield of β-phenylpropionic acid. The distillate was treated with a solution of 2 : 4-dinitrophenylhydrazine in hydrochloric acid; the bis-2 : 4-dinitrophenylhydrazone of methylglyoxal, which very slowly separated, formed orange-red crystals from nitrobenzene–toluene, m. p. 300° (lit., m. p. 300°).

*α-(β-p-Methoxyphenylacrylyl)tetronic Acid* (II; R = *p*-MeO·$C_6H_4$).—The condensation of α-acetyltetronic acid with anisaldehyde was effected as in the case of benzaldehyde. The diluted reaction mixture was extracted with warm ethyl acetate, and the organic layer shaken with N-sodium hydroxide, from which the crude condensation product was precipitated with hydrochloric acid. Crystallisation from 50% alcohol (charcoal) and then light petroleum (b. p. 80—100°) gave bright yellow needles (2% yield), m. p. 164° after softening at 159° (Found: C, 64·5; H, 4·7. $C_{14}H_{12}O_5$ requires C, 64·6; H, 4·7%). Its colourless sodium salt is sparingly soluble in water.

*α-(β-Styrylacrylyl)tetronic Acid* (II; R = Ph·CH:CH).—α-Acetyltetronic acid was condensed with cinnamaldehyde as in the case of benzaldehyde. After cooling, the product was collected, washed with acetic acid and then alcohol, dissolved in a large volume of warm N/20-sodium hydroxide (charcoal), filtered, precipitated with hydrochloric acid, collected, and crystallised from a mixture of equal volumes of acetic acid and alcohol, being obtained in orange-yellow spherulitic growths of minute needles (21% yield), m. p. 178—182° (Found: C, 70·1; H, 4·8. $C_{15}H_{12}O_4$ requires C, 70·3; H, 4·7%). Its solutions in alkalis are almost colourless.

*α-(δ-Phenylvaleryl)tetronic Acid* (III; R = Ph·$CH_2$·$CH_2$).—The preceding acid (1 g.) in N-sodium hydroxide and alcohol (25 c.c.) was shaken in hydrogen in presence of Raney nickel, rather less than 2 mols. of hydrogen being absorbed. The product obtained by acidification of the filtrate was dissolved in boiling water (220 c.c.), filtered, and concentrated hydrochloric acid (3 c.c.) added; the product which separated from the cooled solution crystallised from light petroleum (b. p. 60—80°) in very pale yellow, flat needles (0·33 g.), m. p. 81·5—82·5° (Found: C, 68·8; H, 6·2. $C_{15}H_{16}O_4$ requires C, 69·2; H, 6·2%). The *acid* is slightly soluble in water and is thrown out on the addition of mineral acid. The aqueous solution gives an orange precipitate with ferric chloride.

*α-(β-2-Furylacrylyl)tetronic Acid* (II; R = $C_4H_3O$).—α-Acetyltetronic acid (7 g.) was condensed, as in the previous cases, with furfuraldehyde (1 mol.). The mixture was heated for 2 hours and stirred into water (400 c.c.), and the product collected, washed, dissolved in benzene (50 c.c.), and extracted into dilute sodium hydroxide solution. After treatment with ether and charcoal, the alkaline solution was acidified, and the product collected, washed, dried (3·6 g.), and crystallised from light petroleum (b. p. 80—100°), giving orange-yellow, microcrystalline spherulitic growths, m. p. 146—148° (Found: C, 59·9; H, 3·7. $C_{11}H_8O_5$ requires C, 60·0; H, 3·6%). Solutions of the *substance* in alkali are almost colourless.

*α-(β-2-Tetrahydrofurylpropionyl)tetronic Acid* (III; R = $C_4H_7O$).—The above acid (1 g.) in N-sodium hydroxide (20 c.c.) and Raney nickel was shaken in hydrogen for 20 hours. Absorption of hydrogen was smooth and ceased after 3 mols. had been taken up. The filtered solution was acidified, filtered, and extracted continuously with ether for 8 hours; the extracts gave a solid product, which separated from light petroleum (b. p. 60—80°) in colourless flakes (0·3 g.), m. p. 73·5—74° (Found: C, 58·6; H, 6·1. $C_{11}H_{14}O_5$ requires C, 58·4; H, 6·2%). The *substance* gives an orange colour with aqueous ferric chloride.

*α-Acetamidotetronic Acid* [With Miss E. J. Macnair].—To α-acetyltetronic acid oxime (7·5 g.; Benary, *Ber.*, 1910, **43**, 1068), dissolved in phosphorus trichloride (25 g.), was slowly added phosphorus pentachloride (4·2 g.) with cooling in ice. After ½ hour at room temperature the phosphorus trichloride was removed in the cold under diminished pressure, and the dark product added to ice; the pale yellow crystals which separated from the solution on cooling were collected, washed, and crystallised twice from alcohol. A further amount of material was obtained by repeated extraction of the aqueous solution with ether. The total yield of the pure product, which crystallised in small needles, m. p. 170°, was 2·1 g. (Found: C, 46·0; H, 4·4; N, 8·7. $C_6H_7O_4N$ requires C, 45·9; H, 4·5; N, 8·8%).

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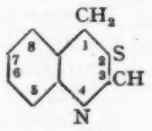
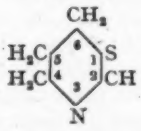
# 63. *Thiazinocyanines. Part II. Cyanines containing the Dihydro-1 : 3-thiazine Nucleus.*

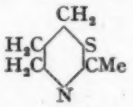
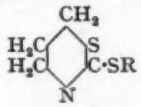
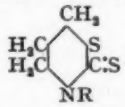
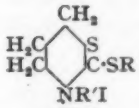
By Frances M. Hamer and Russell J. Rathbone.

2-Thioldihydro-1 : 3-thiazine was converted into its methyl and ethyl derivatives. Alkiodides of these were condensed with heterocyclic quaternary ammonium salts to give simple cyanines of various new types. 2-Methyldihydro-1 : 3-thiazine was prepared by a new method and its alkiodides were used as the starting point for synthesising symmetrical and unsymmetrical carbocyanines. Absorption maxima of the various dyes were compared. The effect of replacing the thiazoline by the dihydro-1 : 3-thiazine nucleus is to produce a bathochromic shift.

After carbocyanines containing the 2 : 4-benzthiazine nucleus (I) had been prepared by use of derivatives having a reactive group in the 3-position (Beilenson and Hamer, J., 1942, 98), the idea of preparing cyanines containing the simple dihydro-1 : 3-thiazine nucleus (II) followed (named originally "Penthiazolin" by

(I.)  (II.) 

Gabriel and Lauer, *Ber.*, 1890, **23**, 87). For cyanine syntheses the 2-methyl derivative (III) and 2-thiol-derivative (IV; R = H), which are known, seemed promising starting points.

 (III.)  (IV.)  (V.)  (VI.)

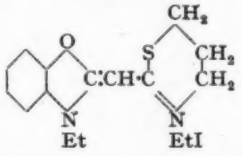
The synthesis of the 2-thiol derivative was effected from αγ-dibromopropane through the stages phthalo-γ-bromopropylimide, γ-bromopropylamine hydrobromide (Gabriel and Weiner, *Ber.*, 1888, **21**, 2669; cf. Putochin, *Ber.*, 1926, **59**, 625), and condensation of this with carbon disulphide in the presence of sodium hydroxide (Gabriel and Lauer, *Ber.*, 1890, **23**, 87). Modifications of the original processes were usefully effected.

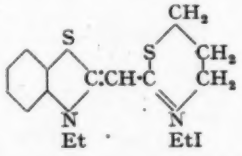
By the action of alkali and alkyl sulphate on the 2-thiol compound (IV; R = H), *2-methylthiodihydro-1 : 3-thiazine* (IV; R = Me) and *2-ethylthiodihydro-1 : 3-thiazine* (IV; R = Et) were prepared. By use of methyl or ethyl iodide the former base was converted into its *methiodide* (VI; R = R′ = Me), and the latter base into its *ethiodide* (VI; R = R′ = Et). During the action of ethyl iodide on the former base, a rearrangement, discussed more fully below, took place, resulting in 2-ethylthiodihydro-1 : 3-thiazine methiodide (VI; R = Et, R′ = Me). By the action of pyridine on 2-methylthiodihydro-1 : 3-thiazine methiodide (cf. Kendall, B.P. 475,647/1936), it was converted into *2-thio-3-methyltetrahydro-1 : 3-thiazine* (V; R = Me), which gave a methiodide. *2-Thio-3-ethyltetrahydro-1 : 3-thiazine* (V; R = Et) was similarly prepared and, from it, an ethiodide.

Following the work of Morton and Stubbs on benzthiazole derivatives (J., 1939, 1321), three ultra-violet absorption spectra in the present series were photographed, with the following results. In methyl-alcoholic solutions the methylthio-base (IV; R = Me) did not give a characteristic absorption band, but the thio-*N*-methyl-base (V; R = Me) and the 2-thiol compound itself each gave a definite and similar absorption curve. From analogy with other series, it may be taken for granted that the constitutions of the two methyl derivatives are as represented by formulæ (IV) and (V), and the evidence of the absorption spectra now indicates that, as in the benzthiazole group (Morton and Stubbs, *loc. cit.*), the "2-thiol-compound" is really a thione, *i.e.*, it has the constitution (V; R = H) rather than (IV; R = H), being *2-thiotetrahydro-1 : 3-thiazine*.

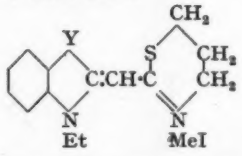
Although Mills, Clark, and Aeschlimann regarded the constitution of the alkiodides of 2-thio-3-methyldihydrobenzthiazole as evident (J., 1923, **123**, 2362), alternatively a sulphonium structure has been formulated (I.G. Farbenind. A.-G., B.P. 423,792/1932). Up till now there has apparently been no direct comparison of the quaternary salt prepared from an alkylthio-compound with the corresponding salt prepared from the thio-*N*-alkyl-compound. The crude methiodide of (IV; R = Me) had a fairly satisfactory iodine value, but was hygroscopic and difficult to recrystallise; it had a rather lower m. p. than the easily recrystallised methiodide of (V; R = Me) (and moreover their mixed m. p. was again slightly lowered), but two recrystallisations brought its m. p. up to that of the methiodide prepared from (V; R = Me) and their mixed m. p. was now the same. Similar results were obtained with the pair of ethiodides. The very considerable drop in yield which occurred on recrystallising each of the two alkiodides prepared from (IV; R = Me or Et) renders our proof of identity incomplete. Our attempt to complete it by photographing ultra-violet absorption spectra of the crude methiodide prepared from the methylthio-compound and of the recrystallised methiodide prepared from the thio-*N*-methyl-compound was inconclusive, because in neither case was a characteristic absorption curve obtained. Sometimes very poor yields of dyes were obtained when the ether-washed alkiodides prepared from (IV; R = Me or Et) were used as starting points, so that preparation of pure alkiodides, either by recrystallisation or by passing through (V), is desirable.

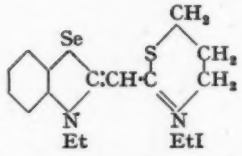
We prepared methincyanines containing the dihydro-1 : 3-thiazine nucleus by condensing quaternary salts such as (VI; R = R′) with other quaternary salts containing a reactive methyl group in absolute alcoholic solution, with anhydrous potassium carbonate or triethylamine as condensing agent. Thus ether-washed 2-ethylthiodihydro-1 : 3-thiazine ethiodide with 2-methylbenzoxazole ethiodide gave (VII) but only in 4% yield; by use of purified 2-methylthiodihydro-1 : 3-thiazine methiodide the 6 : 7-benz-derivative of a methylethyl analogue of (VII) was obtained in 12% yield.

(VII.) 

(VIII.) 

Condensation of the quaternary salts (VI; R = R′) proceeded more smoothly with quaternary salts of 2-methylbenzthiazole, and of its derivatives, than it did with the corresponding benzoxazole salts. Thus there were prepared (VIII), its 4 : 5- and 6 : 7-benz- and 5-chloro-derivatives, its dimethyl analogue and the 4 : 5- and 6 : 7-benz-derivatives of that, also 6-chloro- and 6-acetamido-derivatives of a methylethyl analogue of (VIII). The yields in these nine preparations varied from 35—67%, lying between 60% and 67% in those cases where the salt (VI) had been purified. When the quaternary salt prepared by the action of ethyl iodide on (IV; R = Me) was condensed with 2-methylbenzthiazole ethiodide, the product proved to be, not (VIII), but its methylethyl analogue (IX; Y = S): this was established by an iodine determination and by m. p. determinations, a genuine specimen of (IX; Y = S) having been synthesised from (VI; R = R′ = Me) and 2-methylbenzthiazole ethiodide.

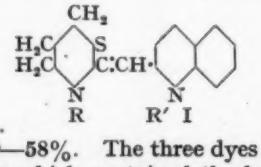
(IX.) 

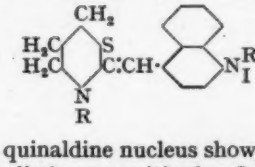
(X.) 

By condensing 2-methylbenzselenazole ethiodide with the ethiodide of 2-ethylthiodihydro-1 : 3-thiazine and with the methiodide of 2-methylthiodihydro-1 : 3-thiazine, respectively, there were obtained (X) and its methylethyl analogue (IX; Y = Se), the yields being 50% and 52%. Condensation of 2-methylbenzselenazole ethiodide with the quaternary salt prepared by the action of ethyl iodide on (IV; R = Me) gave the methylethyl cyanine (IX; Y = Se) and not the diethyl cyanine (X), as shown by iodine and m. p. determinations. From the two anomalous results, in the benzthiazole and benzselenazole series, it is concluded that, when ethyl iodide acts on (IV; R = Me), an interchange of alkyl groups takes place, giving 2-ethylthiodihydro-1 : 3-thiazine methiodide. The same type of rearrangement occurs when ethyl iodide acts upon 2-methylthioquinoline (Beilenson and Hamer, J., 1939, 143) and 2-methylthiobenzthiazole (Sexton, J., 1939, 470). Because of the possibility of such behaviour, the recommendation (Hamer, J., 1940, 799) that, in syntheses by means of quaternary salts of alkylthio-compounds, the salts should be so chosen that the alkyl of the alkylthio-group is identical with the alkyl of the ester used for salt formation, has been adopted in the present work.

Absorption curves of methyl-alcoholic solutions of the new cyanines were plotted. The solution of (VII) is colourless with an absorption maximum at 3660 A. That of the 6 : 7-benz-derivative of its methylethyl analogue is pale yellow, with the maximum 110 A. nearer to the red end of the spectrum. Neither of these compounds sensitised a gelatinochloride photographic emulsion. On passing from the oxa-compound (VII) to the thia-compound (VIII), the maximum undergoes a bathochromic shift of 280 A. Passing from (VIII) to its 4 : 5- and 6 : 7-benz-derivatives, the shifts in the same direction are 160 and 210 A., respectively. Passing from thia- to selena-compound (VIII—X), the shift is only 60 A.

By condensing the alkiodide (VI; R = R′ = Me or Et) with quinaldine alkiodide or lepidine alkiodide, respectively, there were obtained three dyes of type (XI) and three dyes of type (XII), the yields varying from

(XI.) 

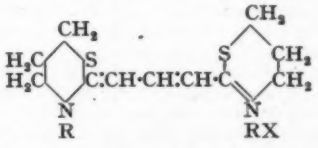
(XII.) 

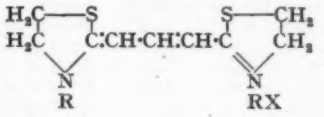
27—46% and from 30—58%. The three dyes containing the quinaldine nucleus showed little or no sensitising action, but with those which contained the lepidine nucleus all three sensitised. On passing from the thia-thiazinocyanine (VIII) to the thiazino-2′-cyanine (XI; R = R′ = Et), the effect of replacing the benzthiazole by the 2-quinoline nucleus was to cause the absorption maximum to shift 510 A. towards the region of longer wave-length; on passing from this thiazino-2′-cyanine to the thiazino-4′-cyanine (XII; R = R′ = Et), the effect of replacing the 2-quinoline nucleus by the 4-quinoline nucleus was to cause a bathochromic shift of 300 A.

2-Methyldihydro-1 : 3-thiazine (III) was prepared by Pinkus by reaction of thioacetamide with excess of

α-chloro-γ-bromopropane, but the yield was only 10% (*Ber.*, 1893, **26**, 1077). As we were unable materially to improve upon this, we devised a new method: α-amino-γ-hydroxypropane, prepared from phthalo-γ-bromopropylimide by Putochin's method (*Ber.*, 1926, **59**, 625), was converted by acetic acid into *α-acetamido-γ-hydroxypropane*, whence (III) was derived by the action of phosphorus pentasulphide. The last two steps are analogous to Wenker's method of preparing α-acetamido-β-hydroxyethane and 2-methylthiazoline, respectively (*J. Amer. Chem. Soc.*, 1935, **57**, 1079). 2-Methyldihydro-1 : 3-thiazine methiodide and ethiodide were obtained as oils, which could not be solidified but were successfully used in the preparation of carbocyanines.

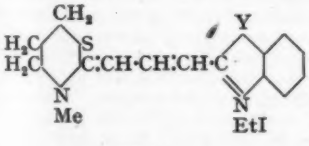
In a French patent which appeared after our work was well under way (I.G. Farbenind. A.-G., F.P. 850,653/1939), 2-methyldihydro-1 : 3-thiazine, prepared by the method of Pinkus, is said to have been converted into its methiodide and ethiodide which, unlike our specimens, were both described as solid substances of definite m. p., and were subjected to various cyanine condensations. For preparing methincyanines containing the simple thiazine nucleus, we prefer the method in which the starting point is the thiol- or thio-derivative (IV or V) rather than the more difficultly accessible methyl derivative (III). According to the French patent, three methincyanines were made by the latter method, namely, (VIII) and two of type (XI; R = Me, R′ = Me and Et, respectively) (examples 5, 3, and 4). The first of these is another point of disagreement, since decomposition at 238° is recorded, whereas we found m. p. 265° (decomp.); the dyes of type (XI) cannot be compared, since they were isolated as perchlorates whereas we made iodides. By condensing the methiodide and ethiodide of (III) with ethyl orthoformate in the presence of pyridine, we prepared a pair of carbocyanines (XIII; R = Me or Et), which proved to be good sensitisers for chloride emulsions. The appropriate example (no. 1) in the French patent approximates in properties to the former of our dyes. That is the only example, out of eight, in which photographic properties are recorded, although the patent purports to deal with photographic sensitisers.

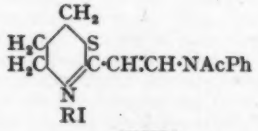
(XIII.) 

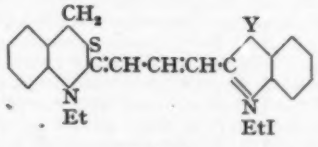

(XIV.)

The thiazinocyanines are particularly interesting as affording the first opportunity of comparing cyanines with others from which they differ only in having an extra member in the ring. In the first paper of this series, it was shown that the absorption maximum undergoes a hypsochromic shift when the benzthiazole nucleus of carbocyanines is replaced by the 2 : 4-benzthiazine nucleus (Beilenson and Hamer, J., 1942, 98). On comparing the present new dyes containing the dihydro-1 : 3-thiazine nucleus with Brooker's dyes containing the thiazoline nucleus (*J. Amer. Chem. Soc.*, 1936, **58**, 662), the shift is found to be in the opposite direction. Thus, on passing from the thiazolinocarbocyanines (XIV; R = Me or Et) to the corresponding thiazinocarbocyanines (XIII; R = Me or Et), the absorption maximum shifts 175 and 165 A., respectively, towards the region of longer wave-length. On passing from the thiazolino-2′-cyanine to the corresponding thiazino-2′-cyanine (XI; R = R′ = Et), there is again a bathochromic shift, but an exact figure cannot be quoted, because the thiazolino-2′-cyanine is peculiar in having two maxima of almost equal intensity. On passing from the thiazolino-4′-cyanine to the corresponding thiazino-4′-cyanine (XII; R = R′ = Et), the absorption maximum shifts 160 A. towards the region of longer wave-length.

By condensing the methiodide of (III) with 2-β-acetanilidovinylbenzoxazole ethiodide and with 2-β-acetanilidovinylbenzthiazole ethiodide, we obtained two unsymmetrical *carbocyanines* (XV; Y = O and S, respec-


(XV.)

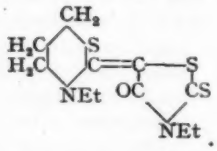

(XVI.)

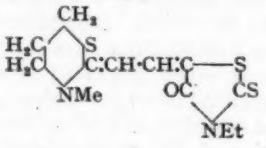

(XVII.)

tively). It has been stated (F.P. 850,653) that the perchlorates of two homologous dyes were prepared from starting points of this type and that one of them was also prepared from (XVI; R = Et) and 2-methylbenzoxazole ethiodide. We prepared 2-β-*anilinovinyldihydro*-1 : 3-*thiazine methiodide* by fusion of 2-methyldihydro-1 : 3-thiazine methiodide with diphenylformamidine, and converted it into 2-β-*acetanilidovinyldihydro*-1 : 3-*thiazine methiodide* (XVI; R = Me) by the action of acetic anhydride. We were, however, unable to obtain (XVI; R = Me) by a method analogous to that recorded for (XVI; R = Et) in the French patent.

An interesting comparison is that between the two dyes (XV; Y = O and S, respectively), containing the dihydro-1 : 3-thiazine nucleus, and the two dyes (XVII; Y = O and S, respectively) containing the 2 : 4-benzthiazine nucleus, which were described by Beilenson and Hamer (*loc. cit.*). On passing from (XV; Y = O) to (XVII; Y = O) the absorption maximum undergoes a bathochromic shift of 300 A., whilst on passing from (XV; Y = S) to (XVII; Y = S) the bathochromic shift is 250 A. The fact that the two dyes of formula (XV) are methylethyl dyes whilst the two of formula (XVII) are diethyl dyes may be discounted, and the above shifts are therefore regarded as those due to replacement of the simple dihydro-1 : 3-thiazine nucleus by the 2 : 4-benzthiazine nucleus.

A couple of dyes, containing the dihydro-1 : 3-thiazine nucleus, but other than cyanines, were prepared by taking advantage of the reactive cyclic methylene group of 3-ethylrhodanine. Condensation of 3-ethylrhodanine with 2-ethylthiodihydro-1 : 3-thiazine ethiodide gave (XVIII) (cf. Kendall, B.P. 426,718/1933; Kodak Ltd., B.P. 450,958/1934), and its condensation with (XVI; R = Me) gave (XIX) (cf. Kendall, B.P. 428,359/1933;

(XVIII.) 

 (XIX.)

Kodak Ltd., *loc. cit.*). On passing from the diethyl compound (XVIII) to the methylethyl compound (XIX), which has an extra dimethin group in the chain, the absorption maximum shifts 830 A. towards the red. Both compounds are photographic sensitisers. For comparison we prepared a dye in which the dihydro-1 : 3-thiazine nucleus of (XIX) was replaced by the thiazoline nucleus. On passing from it to (XIX), the absorption maximum shifted 110 A. into the region of longer wave-length.

## Experimental.

*Phthalo-γ-bromopropylimide.*—αγ-Dibromopropane and potassium phthalimide were heated together with mechanical stirring. Extraction with light petroleum (b. p. 40—60°) gave a 74% yield of the imide (Gabriel and Weiner, *loc. cit.*, obtained 62%) (Found for material dried in a vacuum desiccator: Br, 29·8. Calc. for $C_{11}H_{10}O_2NBr$: Br, 29·8%).

*γ-Bromopropylamine Hydrobromide.*—Gabriel and Weiner's method (*loc. cit.*) was modified by use of acetic acid and by increasing the time of heating: the preceding imide (150 g.), hydrobromic acid (*d* 1·5; 150 c.c.), and acetic acid (75 c.c.) were refluxed for 8 hours. The hot product was poured on ice, the phthalic acid removed, and the filtrate concentrated on the water-bath under reduced pressure. The product was taken up in hot ethyl alcohol (1 c.c. per g.); the substance which crystallised from the filtered solution was ground with ether (yield, 108 g.; 89%) and used for the next stage. After recrystallisation from alcohol and ether (1 c.c. and 4 c.c. per g.), the m. p. was 161—163° (yield, 59%); Gabriel and Weiner give m. p. 162°. For analysis, the salt was dried to constant weight in a vacuum at 60—80°, which method of drying was always used, except where otherwise stated (Found: Br, 72·8. Calc. for $C_3H_8NBr_2$: Br, 73·0%).

2-*Thiotetrahydro*-1 : 3-*thiazine* (or 2-*Thioldihydro*-1 : 3-*thiazine*).—γ-Bromopropylamine hydrobromide (35 g.; 1 mol.), in a flask cooled with ice, was treated with excess of carbon disulphide (28 c.c.; 3 mols.) and stirred while an ice-cold solution of sodium hydroxide (14·8 g.; 2·2 mols.) in water (93 c.c.) was run in; stirring was then continued for 45 minutes. After standing in the ice-chest overnight, the thio-compound was collected and washed with water (yield, 17·2 g.; 81%). After recrystallisation from absolute alcohol (15 c.c. per g.), the yield was 69% (Found: S, 47·9. Calc. for $C_4H_7NS_2$: S, 48·1%). M. p. 129—131°; Gabriel and Lauer give m. p. 132° (*loc. cit.*). Its methyl-alcoholic solution had an absorption maximum at 2875 A.

2-*Methylthiodihydro*-1 : 3-*thiazine* (IV; R = Me).—Methyl sulphate (13·5 c.c.; 1·5 mols.) was gradually added, with vigorous shaking, to 2-thiotetrahydro-1 : 3-thiazine (24 g.; 1 mol.) dissolved in cold 20% sodium hydroxide solution (270 c.c.). The resultant oil was extracted with ether, dried with sodium sulphate, recovered, and distilled in a vacuum (yield, 13·1 g.; 50%) (Found: S, 43·4. $C_5H_9NS_2$ requires S, 43·6%). B. p. 155—160°/50 mm. Its methyl-alcoholic solution showed no characteristic absorption maximum.

2-*Ethylthiodihydro*-1 : 3-*thiazine* (IV; R = Et).—2-Thiotetrahydro-1 : 3-thiazine (8 g.; 1 mol.) was suspended in 20% sodium hydroxide solution (20 c.c.; 1·7 mols.), and ethyl sulphate (8 c.c.; 2 mols.) added, with shaking. The mixture was then heated on the water-bath for 30 minutes, with occasional shaking, and after 12 hours the oil was extracted with ether and twice vacuum-distilled (yield of colourless oil, 37%) (Found: S, 39·7. $C_6H_{11}NS_2$ requires S, 39·8%). B. p. 145—150°/40 mm.

2-*Methylthiodihydro*-1 : 3-*thiazine Methiodide* (VI; R = R′ = Me).—2-Methylthiodihydro-1 : 3-thiazine (10·95 g.; 1 mol.) and methyl iodide (9·2 c.c.; 2 mols.) were left in a sealed tube at room temperature for 3 days. The yield of ether-washed, white, hygroscopic solid, m. p. 121°, was 83% (17·9 g.). After two recrystallisations from ethyl alcohol (1·5 c.c. per g.) (yield, 36%) the m. p. was constant at 132—133° (Found for the crude product after drying in a vacuum desiccator: I, 43·6. Found for the twice recrystallised product: I, 43·9. $C_6H_{12}NIS_2$ requires I, 43·9%). By the second method, 2-thio-3-methyltetrahydro-1 : 3-thiazine (2 g.; 1 mol.) and methyl iodide (1·3 c.c.; 1·5 mols.) were sealed up together in a tube; solution occurred, with rise of temperature, and the liquid subsequently solidified to a white mass. After standing for a few hours at room temperature, the product was ground with absolute ether, being obtained in 94% yield (3·9 g.), m. p. 129°. It was recrystallised from ethyl alcohol (1·5 c.c. per g.) and obtained in 75% yield, m. p. 132° (Found for material dried in a vacuum desiccator: I, 43·95%). The mixed m. p. of the crude methiodides prepared by the two different methods was 115—116°, but the mixed m. p. of recrystallised samples was 132°.

2-*Ethylthiodihydro*-1 : 3-*thiazine Ethiodide* (VI; R = R′ = Et).—After 2-ethylthiodihydro-1 : 3-thiazine (1 mol.) and ethyl iodide (2 mols.) had been kept in a sealed tube at room temperature for 3 days, this was heated at 100° for 8 hours. The viscous liquid solidified when ground with absolute ether (78% yield). After recrystallisation from ethyl alcohol (1·2 c.c. per g.) the yield was 19% (Found for the recrystallised salt dried in a vacuum desiccator: I, 40·1. $C_6H_{14}NIS_2$ requires I, 40·0%). M. p. 98°. By the second method, 2-thio-3-ethyltetrahydro-1 : 3-thiazine (1·6 g.; 1 mol.) and ethyl iodide (1·1 mols.) were heated together in a sealed tube at 100° for 30 minutes, and the product ground with absolute ether. A 98% yield of almost colourless solid was obtained and after recrystallisation from ethyl alcohol (1·5 c.c. per g.) the yield was 50% (Found for the ether-washed product after drying in a vacuum desiccator: I, 40·15. Found for the recrystallised product: I, 40·1%). M. p. 99°.

2-*Ethylthiodihydro*-1 : 3-*thiazine Methiodide* (VI; R = Et, R′ = Me).—2-Methylthiodihydro-1 : 3-thiazine (IV; R = Me) (1 mol.) and ethyl iodide (2 mols.), when kept in a sealed tube at room temperature for 3 days, deposited an oil. After heating at 60° for 16 hours and at 100° for 24 hours, the product was brown and viscous, but refused to solidify when cooled and washed with absolute ether. Its constitution was inferred from analyses of two of its condensation products.

2-*Thio*-3-*methyltetrahydro*-1 : 3-*thiazine* (V; R = Me).—2-Methylthiodihydro-1 : 3-thiazine methiodide (10·5 g.) was boiled with pyridine (36 c.c.) for 20 minutes, and the solution poured into water (180 c.c.) and concentrated to one-third under reduced pressure. The product crystallised on cooling and was recrystallised from ethyl alcohol (26 c.c.), the yield

of colourless needles, m. p. 88°, being 71% (4·03 g.) (Found for material dried in a vacuum at 50° : S, 43·8. $C_5H_9NS_2$ requires S, 43·6%). Its methyl-alcoholic solution had an absorption maximum at 2825 A.

*2-Thio-3-ethyltetrahydro-1 : 3-thiazine* (V; R = Et).—2-Ethylthiodihydro-1 : 3-thiazine ethiodide (19 g.) was boiled with pyridine (30 c.c.) for an hour, and the solution poured into water. The resultant oil solidified (67% yield). On recrystallisation from ethyl alcohol (4 c.c. per g.), the yield of almost colourless crystals, m. p. 68°, was 54% (Found for material dried in a vacuum desiccator : S, 40·0. $C_6H_{11}NS_2$ requires S, 39·8%).

*α-Amino-γ-hydroxypropane.*—This was prepared by Putochin's method (*loc. cit.*). The process of taking the aqueous solution down to dryness was repeated three times instead of twice and was carried out under reduced pressure by heating in an oil-bath. The yield was 75%, b. p. 184—186°, whereas he records an almost 90% yield.

*α-Acetamido-γ-hydroxypropane.*—α-Amino-γ-hydroxypropane (77 g.; 1 mol.) was stirred while acetic acid (65 c.c.; 1 mol.) was slowly added. The liquid was heated, in order to drive off the liberated water, until its temperature was 200°. The yield was 87% (106 g.) and this crude product was used for the next stage of the synthesis (Found : N, 12·15. $C_5H_{11}O_2N$ requires N, 12·0%).

*2-Methyldihydro-1 : 3-thiazine* (III).—A mixture of α-acetamido-γ-hydroxypropane (32 g.; 5 mols.) and phosphorus pentasulphide (16 g.; 1·1 mols.) was heated to start reaction. When this abated, heat was again applied, and distillation continued up to 190°. The resultant oil was distilled; the fraction, b. p. 165—175°, amounted to a 20% yield (13·5 g. from two such batches). Pinkus gives b. p. 173°/757 mm. (*loc. cit.*).

*2-Methyldihydro-1 : 3-thiazine Methiodide.*—2-Methyldihydro-1 : 3-thiazine (11·2 g.; 1 mol.) and methyl iodide (12 c.c.; 1·5 mols.) were mixed; reaction then occurred. After standing in a closed vessel at room temperature for 3 hours, the resultant yellow oil was washed with absolute ether, being obtained in 66% yield (16 g.).

*2-Methyldihydro-1 : 3-thiazine Ethiodide.*—2-Methyldihydro-1 : 3-thiazine (2 g.; 1 mol.) and ethyl iodide (3 c.c.; 2 mols.) were heated together in a sealed tube at 100° for 3 hours. After being washed with absolute ether, the yield of viscous salt was 75%.

*2-β-Anilinovinyldihydro-1 : 3-thiazine Methiodide.*—2-Methyldihydro-1 : 3-thiazine methiodide (7 g.; 1 mol.) and diphenylformamidine (6·1 g.; 1 mol.) were heated together in an oil-bath at 140° for 10 minutes, with frequent stirring. The melt was taken up in hot spirit (12 c.c.); on cooling, bright yellow crystals, m. p. 165°, separated in 56% yield (5·6 g.) (Found : I, 35·45. $C_{12}H_{17}N_2IS$ requires I, 35·25%).

*2-β-Acetanilidovinyldihydro-1 : 3-thiazine Methiodide* (XVI; R = Me).—The preceding compound (1 g.) was boiled for 10 minutes with acetic anhydride (5 c.c.). When the solution was cooled in ice, a yellow solid crystallised. After washing with ether, the yield was 18% (0·2 g.) (Found : I, 31·7. $C_{15}H_{19}ON_2IS$ requires I, 31·6%) and m. p. 180—185°.

*[2-(3-Ethyldihydro-1 : 3-thiazine)][2-(3-ethylbenzoxazole)]methincyanine Iodide* (VII).—Ether-washed 2-ethylthiodihydro-1 : 3-thiazine ethiodide (5·1 g.; 1 mol.), 2-methylbenzoxazole ethiodide (4·6 g.; 1 mol.), triethylamine (3·9 c.c.; 1 mol.), and absolute alcohol (20 c.c.) were boiled together, with stirring, for 2 minutes. The product was collected, washed with water, and crystallised from absolute alcohol (50 c.c. per g.), but the yield was only 4% (Found : I, 30·3. $C_{14}H_{21}ON_2IS$ requires I, 30·5%). The pale yellow crystals had m. p. 260° (decomp.) and gave an almost colourless methyl-alcoholic solution with absorption maximum at 3660 A.

*[2-(3-Methyldihydro-1 : 3-thiazine)][2-(3-ethyl-6 : 7-benzbenzoxazole)]methincyanine Iodide.*—Purified 2-methylthiodihydro-1 : 3-thiazine methiodide (1·83 g.; 1 mol.), 2-methyl-6 : 7-benzbenzoxazole ethiodide (2·15 g.; 1 mol.), triethylamine (1·3 c.c.; 1·5 mols.), and absolute alcohol (12 c.c.) were boiled together, with stirring, for 7 minutes. The product was collected, washed with water and ether (0·47 g. obtained), and crystallised from absolute alcohol (30 c.c.). The yield was 12% (0·35 g.) (Found : I, 27·9. $C_{19}H_{21}ON_2IS$ requires I, 28·1%). The pale pink solid, m. p. 264° (decomp.), gave a methyl-alcoholic solution with absorption maximum at 3770 A.

*[2-(3-Methyldihydro-1 : 3-thiazine)][2-(3-methylbenzthiazole)]methincyanine Iodide.*—This was prepared by boiling together ether-washed 2-methylthiodihydro-1 : 3-thiazine methiodide (1·45 g.; 1 mol.), 2-methylbenzthiazole methiodide (1 mol.), anhydrous potassium carbonate (180-mesh; 1·2 mols.), and absolute alcohol for 30 seconds, with hand stirring. After recrystallisation of the water-washed product from methyl alcohol (70 c.c. per g.), the yield was 55% (Found : I, 31·3. $C_{14}H_{17}N_2IS_2$ requires I, 31·4%). M. p. 283° (decomp.). A methyl-alcoholic solution of the pinkish crystals had its absorption maximum at 3920 A. The substance weakly sensitised a gelatinochloride photographic emulsion, giving a maximum at about 4150.

*[2-(3-Ethyldihydro-1 : 3-thiazine)][2-(3-ethylbenzthiazole)]methincyanine Iodide* (VIII).—This was prepared from recrystallised 2-ethylthiodihydro-1 : 3-thiazine ethiodide and 2-methylbenzthiazole ethiodide, being obtained in 67% yield after recrystallisation from methyl alcohol (33 c.c. per g.) (Found : I, 29·4. $C_{16}H_{21}N_2IS_2$ requires I, 29·4%). The buff solid had m. p. 265° (decomp.), whereas a specimen of this constitution has been described elsewhere (F.P. 850,653/1939, ex. 5) as decomposing at 238°. A methyl-alcoholic solution had its absorption maximum at 3940 A. The cyanine weakly sensitised a chloride emulsion, the maximum effect occurring at 4200 A. and sensitisation extending to 4500.

*[2-(3-Methyldihydro-1 : 3-thiazine)][2-(3-methyl-4 : 5-benzbenzthiazole)]methincyanine Iodide.*—This was prepared from ether-washed 2-methylthiodihydro-1 : 3-thiazine methiodide and 2-methyl-4 : 5-benzbenzthiazole methiodide. Heating at the reaction stage was for 3 minutes and that was the time of heating in the following condensations, except where otherwise stated. After recrystallising from methyl alcohol (65 c.c. per g.) the yield was 54% (Found : I, 27·9. $C_{18}H_{19}N_2IS_2$ requires I, 27·9%). The greenish crystals had m. p. 226° (decomp.). They weakly sensitised a chloride emulsion up to 4700 A., with a maximum at 4300.

*[2-(3-Methyldihydro-1 : 3-thiazine)][2-(3-methyl-6 : 7-benzbenzthiazole)]methincyanine Iodide.*—The 6 : 7-benz-derivative was prepared by use of 2-methyl-6 : 7-benzbenzthiazole methiodide. After recrystallisation from methyl alcohol (80 c.c. per g.), the yield was 58% (Found : I, 27·8. $C_{18}H_{19}N_2IS_2$ requires I, 27·9%). The greenish-yellow crystals had m. p. 267° (decomp.). The substance sensitised a chloride emulsion moderately to beyond 4700 A., the maximum lying at 4300.

*[2-(3-Ethyldihydro-1 : 3-thiazine)][2-(3-ethyl-4 : 5-benzbenzthiazole)]methincyanine Iodide.*—This was prepared from ether-washed 2-ethylthiodihydro-1 : 3-thiazine ethiodide (1·59 g.) and 2-methyl-4 : 5-benzbenzthiazole etho-*p*-toluenesulphonate, with potassium carbonate in alcohol, the time of heating being 15 minutes, with mechanical stirring. The product was dissolved in hot spirit (75 c.c.) and treated with a hot solution of potassium iodide (3 g.) in water (200 c.c.). The resultant iodide (1·46 g.) was recrystallised from methyl alcohol (50 c.c.) with addition of charcoal (0·5 g.), and was obtained in 35% yield (Found : I, 26·4. $C_{20}H_{23}N_2IS_2$ requires I, 26·3%). The greenish-yellow crystals had m. p. 238° (decomp.). The methyl-alcoholic solution had its absorption maximum at 4100 A. The substance is a fairly good sensitiser for a washed chloride emulsion, the maximum lying at 4350.

*[2-(3-Ethyldihydro-1 : 3-thiazine)][2-(3-ethyl-6 : 7-benzbenzthiazole)]methincyanine Iodide.*—This was prepared as above by use of 2-methyl-6 : 7-benzbenzthiazole etho-*p*-toluenesulphonate, followed by treatment with potassium iodide solution. The dye *iodide* was recrystallised from methyl alcohol (40 c.c. per g.) with addition of charcoal (0·3 g. per g.) and obtained in 43% yield (Found : I, 26·2. $C_{20}H_{23}N_2IS_2$ requires I, 26·3%). The greenish-yellow crystals had m. p. 254° (decomp.). The absorption maximum of the methyl-alcoholic solution was at 4150 A. The substance

is a fairly good sensitiser for a washed chloride emulsion, the maximum lying at 4300 and sensitivity being extended past 4700.

[2-(3-*Ethyldihydro*-1 : 3-*thiazine*)][2-(5-*chloro*-3-*ethylbenzthiazole*)]*methincyanine Iodide*.—Ether-washed 2-ethylthiodihydro-1 : 3-thiazine ethiodide was condensed with 5-chloro-2-methylbenzthiazole ethiodide. After recrystallisation from absolute alcohol (70 c.c. per g.), the yield was 52% (Found : Cl + I, 34·7. $C_{16}H_{20}N_2ClIS_2$ requires Cl + I, 34·8%). The pinkish-yellow crystals had m. p. 251° (decomp.). The absorption maximum is at 3940 A. The salt is a fair sensitiser up to 4600, with a maximum at 4150.

[2-(3-*Methyldihydro*-1 : 3-*thiazine*)][2-(6-*chloro*-3-*ethylbenzthiazole*)]*methincyanine Iodide*.—Crystallised 2-methylthiodihydro-1 : 3-thiazine methiodide was condensed with 6-chloro-2-methylbenzthiazole ethiodide and recrystallisation was from methyl alcohol (150 c.c. per g.). The yield was 64% whether anhydrous potassium carbonate or triethylamine was used as condensing agent (Found : Cl + I, 36·0. $C_{15}H_{18}N_2S_2ClI$ requires Cl + I, 35·9%). The pinkish solid had m. p. 283° (decomp.) and gave an almost colourless methyl-alcoholic solution. It caused extremely slight sensitisation with maximum at 4100 A.

[2-(3-*Methyldihydro*-1 : 3-*thiazine*)][2-(6-*acetamido*-3-*ethylbenzthiazole*)]*methincyanine Iodide*.—Crystallised 2-methylthiodihydro-1 : 3-thiazine methiodide and 6-acetamido-2-methylbenzthiazole ethiodide were similarly condensed. After recrystallisation from methyl alcohol (135 c.c. per g.), the yields were materially the same (63% and 60%) whether potassium carbonate or triethylamine had been used as condensing agent (Found : I, 26·8. $C_{17}H_{22}ON_2IS_2$ requires I, 26·7%). The yellow crystalline substance had m. p. 279° (decomp.). The absorption maximum was at 4000 A. and the sensitising maximum at 4200, the sensitisation extending to 4600.

[2-(3-*Methyldihydro*-1 : 3-*thiazine*)][2-(3-*ethylbenzthiazole*)]*methincyanine Iodide* (IX; Y = S).—This was prepared from ether-washed 2-methylthiodihydro-1 : 3-thiazine methiodide and 2-methylbenzthiazole ethiodide in alcohol in the presence of potassium carbonate, with boiling and mechanical stirring for 10 minutes. The water-washed product was recrystallised from absolute alcohol (33 c.c. per g.) with addition of charcoal (1 g. per g.) to remove a trace of pink impurity. This caused the yield to drop from 74 to 25% (Found : S, 15·6; I, 30·4. $C_{15}H_{19}N_2IS_2$ requires S, 15·3; I, 30·35%). The buff crystals decomposed at 249°. It is a very weak sensitiser with maximum at about 4200 A.

When the salt from 2-methylthiodihydro-1 : 3-thiazine and ethyl iodide was condensed with 2-methylbenzthiazole ethiodide, the washed product, after recrystallisation from absolute alcohol (40 c.c. per g.), was obtained in 76% yield. After a second recrystallisation, an iodine determination indicated that the salt isolated is the above methylethyl, and not the diethyl, derivative (Found : I, 30·2%). This was confirmed by its m. p. and by its mixed m. p. with the above methylethyl compound.

[2-(3-*Ethyldihydro*-1 : 3-*thiazine*)][2-(3-*ethylbenzselenazole*)]*methincyanine Iodide* (X).—This was prepared from recrystallised 2-ethylthiodihydro-1 : 3-thiazine ethiodide and 2-methylbenzselenazole ethiodide, with potassium carbonate in alcohol, being obtained after recrystallisation from absolute alcohol (80 c.c. per g.), with addition of charcoal (0·4 g. per g.), in 50% yield. Iodine in the presence of selenium was determined as described by Hamer (*Analyst*, 1933, **58**, 26) (Found : I, 26·6. $C_{16}H_{21}N_2ISSe$ requires I, 26·5%). The greenish-yellow crystals had m. p. 264° (decomp.). The absorption maximum is at 4000 A. and the sensitising maximum at 4300, sensitivity extending beyond 4500.

[2-(3-*Methyldihydro*-1 : 3-*thiazine*)][2-(3-*ethylbenzselenazole*)]*methincyanine Iodide* (IX; Y = Se).—Prepared by condensation of ether-washed 2-methylthiodihydro-1 : 3-thiazine methiodide and 2-methylbenzselenazole ethiodide, this was obtained in 52% yield after recrystallisation from absolute alcohol (75 c.c. per g.) with addition of charcoal (0·3 g. per g.) (Found : I, 27·4. $C_{15}H_{19}N_2ISSe$ requires I, 27·3%). The yellow crystals had m. p. 271° (decomp.). It is a weak sensitiser with maximum at about 4250 A., the action extending to 4600.

When the salt prepared from 2-methylthiodihydro-1 : 3-thiazine and ethyl iodide was condensed with 2-methylbenzselenazole ethiodide, the product, after recrystallisation from absolute alcohol (40 c.c. per g.), resulted in 28% yield and an iodine determination indicated that it is the above methylethyl, and not the diethyl, derivative (Found : I, 27·1%). The m. p. and mixed m. p. were identical with that of the methylethyl cyanine, all taken simultaneously.

[2-(3-*Methyldihydro*-1 : 3-*thiazine*)][2-(1-*methylquinoline*)]*methincyanine Iodide* (XI; R = R′ = Me).—Recrystallised 2-methylthiodihydro-1 : 3-thiazine methiodide (0·97 g.; 1 mol.), quinaldine methiodide (0·95 g.; 1 mol.), triethylamine (0·7 c.c.; 1·5 mols.), and absolute alcohol (10 c.c.) were boiled together, with stirring, for 3 minutes. The product was washed with water and with ether (0·66 g. obtained). After recrystallisation from absolute alcohol (20 c.c.), the yield was 46% (0·61 g.) (Found : I, 31·9. $C_{16}H_{19}N_2IS$ requires I, 31·9%). The vermilion crystals had m. p. 225° (decomp.). Their methyl-alcoholic solution had its principal absorption maximum at 4450 and a weaker one at 4250 A.

[2-(3-*Methyldihydro*-1 : 3-*thiazine*)][2-(1-*ethylquinoline*)]*methincyanine Iodide* (XI; R = Me, R′ = Et).—Prepared from crystallised 2-methylthiodihydro-1 : 3-thiazine methiodide, quinaldine ethiodide, and triethylamine in alcohol, with boiling for 7 minutes, and recrystallised from methyl alcohol (4 c.c. per g.), it was obtained in 27% yield (Found : I, 30·9. $C_{17}H_{21}N_2IS$ requires I, 30·8%). When ether-washed 2-methylthiodihydro-1 : 3-thiazine methiodide was used, the yield was only 3%. The yellow crystals had m. p. 189° (decomp.). The absorption maximum was at 4500 A. with a secondary maximum at 4300. This dye and the preceding one did not sensitise a silver chloride photographic emulsion.

[2-(3-*Ethyldihydro*-1 : 3-*thiazine*)][2-(1-*ethylquinoline*)]*methincyanine Iodide* (XI; R = R′ = Et).—Prepared from crystallised 2-ethylthiodihydro-1 : 3-thiazine ethiodide, quinaldine ethiodide, and triethylamine in alcohol, with boiling for 5 minutes, and recrystallised from methyl alcohol (3 c.c. per g.), the orange-yellow dye was obtained in 31% yield, but the result was negative when the ether-washed 2-ethylthiodihydro-1 : 3-thiazine ethiodide was used (Found : I, 29·9. $C_{18}H_{23}N_2IS$ requires I, 29·8%). M. p. 169° (decomp.). The principal absorption maximum was at 4450 A. with a secondary one at 4250. The dye was almost inert photographically but there was a trace of conferred sensitivity at 4700 A.

[2-(3-*Methyldihydro*-1 : 3-*thiazine*)][4-(1-*methylquinoline*)]*methincyanine Iodide* (XII; R = R′ = Me).—Prepared from crystallised 2-methylthiodihydro-1 : 3-thiazine methiodide, lepidine methiodide, and triethylamine in alcohol, with 5 minutes' boiling, the product, after being washed with ether and water and recrystallised from methyl alcohol (1·5 c.c. per g.), was obtained in 38% yield. After a second recrystallisation from ethyl alcohol (15 c.c. per g.), the yield was 30% (Found : I, 32·0. $C_{16}H_{19}N_2IS$ requires I, 31·9%). The bright orange crystalline substance had m. p. 164—165° (decomp.). It sensitised a chloride emulsion weakly to 5300 A. with a maximum at 4950. Both this and the next dye, in methyl-alcoholic solution, had the absorption maximum at 4800.

[2-(3-*Methyldihydro*-1 : 3-*thiazine*)][4-(1-*ethylquinoline*)]*methincyanine Iodide* (XII; R = Me, R′ = Et).—This was prepared from crystallised 2-methylthiodihydro-1 : 3-thiazine methiodide with lepidine ethiodide and triethylamine in alcohol, and recrystallised twice from ethyl alcohol (10 c.c. per g.), being obtained in 58% yield (Found : I, 30·7. $C_{17}H_{21}N_2IS$ requires I, 30·8%). The dull orange crystals had m. p. 202° (decomp.). It sensitised weakly to 5300 A. with a maximum at 4900.

[2-(3-*Ethyldihydro*-1 : 3-*thiazine*)][4-(1-*ethylquinoline*)]*methincyanine Iodide* (XII; R = R′ = Et).—Prepared from lepidine ethiodide, crystallised 2-ethylthiodihydro-1 : 3-thiazine ethiodide, and triethylamine in alcohol, the washed product, after recrystallisation from ethyl alcohol (17 c.c. per g.), was obtained in 47% yield (Found : I, 29·7. $C_{18}H_{23}N_2IS$ requires I, 29·8%). The orange crystals melted at 191° (decomp.). The absorption maximum of a methyl-

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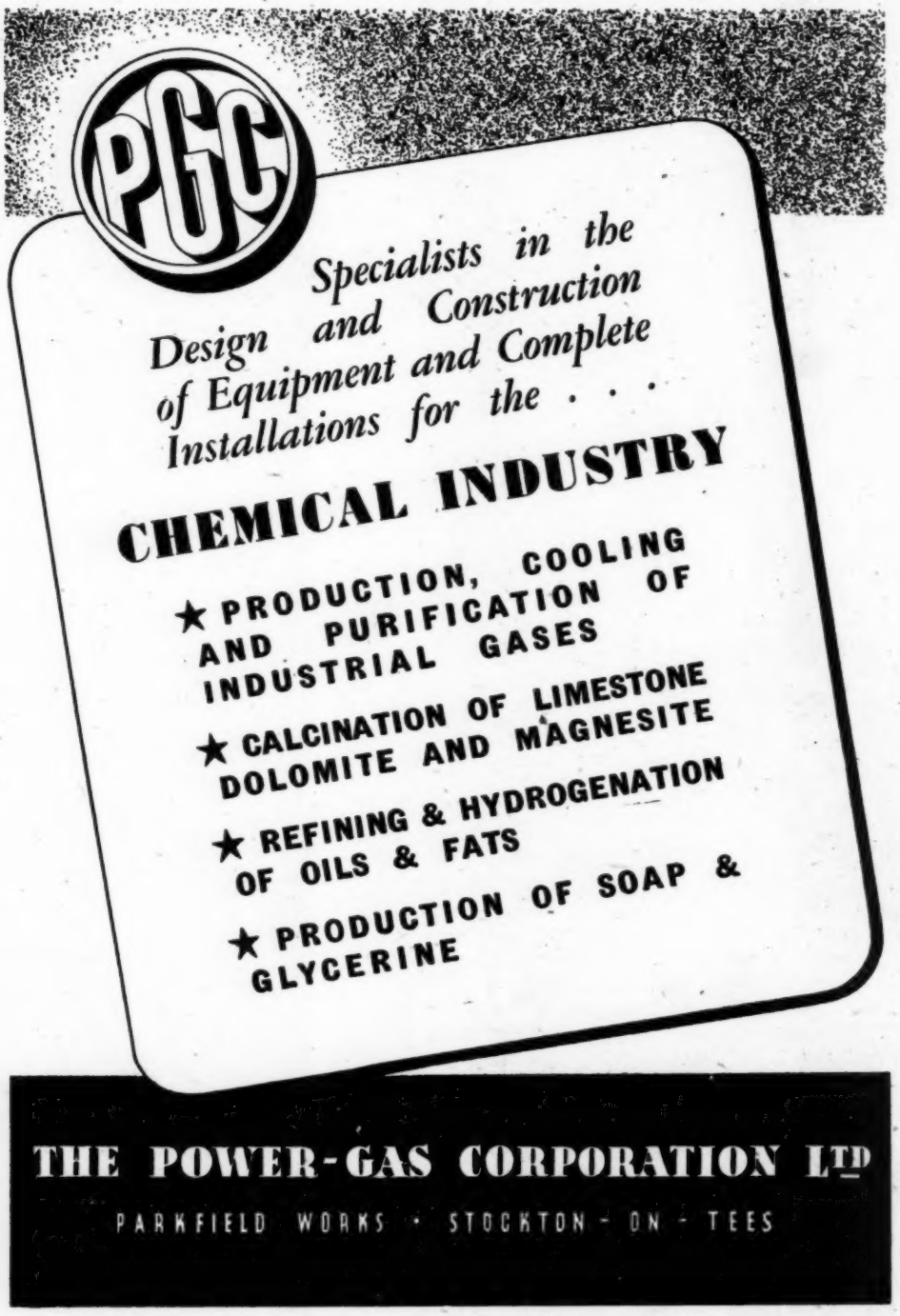
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